A COMPARISON OF LOW DENSITY LIPOPROTEIN CHOLESTEROL CONCENTRATION ESTIMATION BY THE ROCHE DIAGNOSTICS DIRECT MEASUREMENT AND THE FRIEDEWALD METHODS: COMPARISON WITH OTHER LIPIDIC CARDIOVASCULAR RISK ESTIMATION BIOMARKERS IN ADULT PATIENTS' SERA IN WINDHOEK, NAMIBIA

A THESIS SUBMITTED IN FULFILMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN BIOCHEMISTRY

OF

THE UNIVERSITY OF NAMIBIA

BY

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APRIL 2019

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ABSTRACT

The primary target for atherosclerosis associated cardiovascular (AACV) risk prediction and treatment assessment is the lipid profile fraction of low density lipoprotein cholesterol (LDLC). The routinely used Friedewald method for LDLC estimation has an unacceptably high analytical error. In this study, patient sera LDLC was estimated using the Friedewald method (cLDLC) and the Roche Diagnostics direct homogeneous method (dLDLC). Serum lipid calculations of non-HDLC, HDLC/LDLC ratios, and sdLDLC were made. A comparison of the cLDLC and the dLDLC methods in the estimation of the LDLC and the classification of the sera into CV risk categories using the cLDLC, dLDLC, non-HDLC, HDLC/LDLC ratios, and sdLDLC was done. Upon using dLDLC in place of cLDLC, 41 out of 121 (34%) serum samples had their CV risk categories changed. This corresponds to a clinical discordance of 34%. Forty of the 41 CV risk category changes were in the form of a decrease by one CV risk category. Also, many other samples showed a decrease in LDLC estimation which did not result in change in CV risk category. The paired t test calculations showed that the means of the cLDLC method and the dLDLC method were significantly different at $p < 0.01$. Upon using non-HDLC in place of cLDLC, 39 out of 121 (32%) serum samples had their CV risk categories changed. This corresponds to a clinical discordance of 32%. Twenty seven of the 39 CV risk category changes were in the form of a decrease by one CV risk category. Upon using non-HDLC in place of dLDLC, 43 out of 121 (36%) serum samples had their CV risk categories changed. This corresponds to a clinical discordance of 36%. Thirty of the 43 CV risk category changes were in the form of an increase by one CV risk category. On all the lipid pairs (cLDLC and dLDLC, cLDLC and non-HDLC, and dLDLC and non-HDLC), the CV risk categories and the paired t test showed that there were significant CV risk category changes and statistically significant result differences between methods although the correlations were very high and in a linear manner. The calculated CV

risk category changes and the statistical outcomes observed in this study show that the cLDLC significantly overestimates the LDLC levels compared to dLDLC, in patient sera with normal or slightly elevated triglycerides (TGs) levels, classifying the patients into higher CV risk categories. On the Low Risk/Risk category analysis, all the methods gave significantly different results, with sdLDLC giving the highest number of sera samples classified into the Risk category. The CV risk classification varies with the method used. Further studies using large samples taken from different ethnic and geographic populations and preferably compared with the Beta Quantification reference method are needed to verify these findings. Institutions in the same geographic location must use the same LDLC method (preferably the dLDLC) for CV risk estimation and treatment assessment. The dLDLC must replace the cLDLC for AACVD risk prediction and treatment assessment. Also, more population based studies must be conducted to establish the best lipid biomarker and lipid panel for CV risk estimation and treatment assessment.

Key words: Atherosclerosis associated cardiovascular risk, direct homogeneous method, Friedewald method, Low-density lipoprotein

LIST OF CONFERENCE(S) PROCEEDINGS

5th Annual Faculty of Science Research Conference “Science today: Global bridge to the future”. 16 November 2017

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ABBREVIATIONS

AACVD	Atherosclerotic Associated Cardiovascular Disease
ACC	American College of Cardiology
AHA	American Heart Association
Apo	Apolipoprotein
CAD	Coronary Artery Disease
CHD	Coronary Heart Disease
cLDLC	calculated Low Density Lipoprotein Cholesterol
CRLMN	Cholesterol Reference Laboratory Method Network
CV	Cardiovascular
CVD	Cardiovascular Disease
dLDLC	direct Low Density Lipoprotein Cholesterol
DT	Drug Therapy
EAS	European Atherosclerosis Society
EFCCLM	European Federation of Clinical Chemistry and Laboratory Medicine
HDL	High Density Lipoprotein
HDLC	High Density Lipoprotein Cholesterol
HDLP	High Density Lipoprotein Particle
IDL	Intermediate Density Lipoprotein

IDLC	Intermediate Density Lipoprotein Cholesterol
IDLP	Intermediate Density Lipoprotein Particle
lbLDL	large buoyant Low Density Lipoprotein
lbLDLC	large buoyant Low Density Lipoprotein Cholesterol
LLD	Lipid Lowering Drug
LLDT	Lipid Lowering Drug Therapy
LDL	Low Density Lipoprotein
LDLC	Low Density Lipoprotein Cholesterol
LDLP	Low Density Lipoprotein Particle
metS	metabolic Syndrome
MoHSS	Ministry of Health and Social Services
NCEP	National Cholesterol Education Program
NICE	National Institute for Health and Care Excellence
NR	No Risk
oxLDL	oxidised Low Density Lipoprotein
oxLDLC	oxidised Low Density Lipoprotein Cholesterol
PAD	Peripheral Artery Disease
PROCAM	Prospective Cardiovascular Munster
PROSPER	Prospective Study of Pravastatin in the Elderly at Risk

R	Risk
sdLDL	small dense Low Density Lipoprotein
sdLDLC	small dense Low Density Lipoprotein Cholesterol
SCORE	Systematic Coronary Risk Evaluation
SREBPs	sterol regulatory element binding proteins
TC	Total Cholesterol
TLC	Therapeutic Lifestyle Change
TGs	Triglycerides
UNAM	University of Namibia
UREC	UNAM Research Council
VLDL	Very Low Density Lipoprotein
VLDLC	Very Low Density Lipoprotein Cholesterol
VLDLP	Very Low Density Lipoprotein Particle
WHO	World Health Organisation

DEFINITION OF TERMS

Atherogenic triad - a dyslipidaemia which is characterised by high TG, low HDLC, high sdLDLC in the presence of normal or low LDLC.

Atherosclerosis – the pathological build-up of cholesterol within the intima of the walls of large and medium sized arteries resulting in endothelial dysfunction and vascular inflammation

Cardiovascular events – the pathological conditions affecting the arteries, namely coronary artery disease, peripheral artery disease, myocardial infarction, and stroke.

CVD - a disease which encompasses any medical condition related to heart and blood arteries.

CV risk estimation – the calculation of a likelihood for one to have a cardiovascular event over a standard period of time.

Dyslipidaemia - a disorder of lipoprotein metabolism resulting in lipoprotein overproduction or deficiency. This disorder manifests as elevation of TC, LDLC, and TG concentrations, and a decrease in the HDLC concentration in blood.

Hypercholesterolemia - the elevation of total cholesterol, LDLC and/or TGs in the blood.

Metabolic Syndrome - a cluster of metabolic conditions that increase CV risk. These conditions include abdominal obesity, hypertriglyceridemia, hyperglycaemia (type 2 diabetes), hypertension, low HDLC, endothelial dysfunction, insulin resistance, pro-inflammatory state, and pro-thrombotic state.

ACKNOWLEDGEMENT

I gratefully acknowledge the assistance of Miss Bertha Amunyela who assisted me with the specimen reception and sorting. I also thank Dr P Kapewangolo and Dr M Mukesi for guiding me through this research. Finally, I am indebted to CLS Pathology Xpress, Nampath, Medical Diagnostic, Glowshot, and Highcare medical laboratories for their help in collecting the suitable blood samples.

DEDICATION

To my family

DECLARATION

I, Clever Muyezi, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

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Name of Student Signature Date

1. INTRODUCTION

1.1 Background of the study

Cardiovascular disease (CVD) due to atherosclerosis of the arterial vessel walls is the leading cause of death in the world [1,2,3,4]. According to the WHO health report of 2008, global estimates of mortality due to CVD were predicted to rise from 17.1 million in 2004 to 23.4 million in 2030 [4,5]. In 2012, the annual direct and indirect health care costs of CVD in Europe were an estimated €192 billion [1]. CVD is also the number one cause of death in sub-Saharan Africa, where adults over the age of 30 are mostly affected [6,7]. Eighty percent of CVD deaths take place in low and middle income countries [3,5,7]. In 2013, an average of 10 people would suffer a stroke and 5 people would have a heart attack every hour in South Africa [6]. In 2014, CVD accounted for 21% (2 940) and 43% (261 440) annual deaths in Namibia and South Africa respectively. In the two countries, the CVD death rates were higher than any other non-communicable disease. In contrast to the global trends whereby the death rate from CVD is increasing, the Namibian and the South African death rates from CVD were constantly dropping from the year 2000 to the year 2012 [8,9].

CVD is a disease associated with the heart and blood vessels.The main clinical entities of CVD are coronary artery disease (CAD), ischaemic stroke, and peripheral artery disease (PAD) [1,3]. Atherosclerosis is the pathological build-up of cholesterol within the intima of the walls of large and medium sized arteries resulting in endothelial dysfunction and vascular inflammation [10]. The prevalence of AACVD is increasing due to over utilization of fats/

increase in dietary fat, a more sedentary life-style, and increase in certain diseases such as diabetes [4,6,10,11].

Blood is frequently analysed for dyslipidaemia in the diagnosis and the subsequent assessment of CV risk [12,13]. The routine assessment of cholesterol as a risk factor in CVD development is done using the lipid profile that commonly includes the measured total cholesterol (TC), triglycerides (TGs), high density lipoprotein cholesterol (HDLC), and calculation of low density lipoprotein cholesterol using the Friedewald method. The LDLC concentration is directly related to the risk of developing CVD [2,13]. Of the various plasma cholesterol fractions, LDLC has the highest proportion of cholesterol and transports about 70% of plasma total cholesterol [14]. The elimination of hypercholesterolemia (primarily the reduction of LDLC) is the single most important benefit against the incidence of AACV events [15]. Expert panels which include the internationally adopted National Cholesterol Education Treatment Programme Adult Treatment Panel III (NCEP ATP III), the European Federation of Clinical Chemistry and Laboratory Medicine, European Atherosclerosis Society (EAS), and the Atherosclerosis Society of South Africa recommend the LDLC serum concentrations as the primary target for classifying adults at risk of developing CVD. Subsequently, the primary goals of lipid lowering drug therapy (LLDT) and the cut-points for initiating treatment are stated in terms of LDLC. Therapy is targeted on lowering LDLC values below the target value, which depends on the number of other risk factors present [14].

Non-High Density Lipoprotein cholesterol (non-HDLC) is a secondary target for atherosclerotic associated cardiovascular (CV) risk diagnosis and drug treatment monitoring [16,17]. Small dense LDLC (sdLDLC) is not yet a target for dyslipidaemia diagnosis and treatment monitoring, but NCEP accepts the predominance of sdLDLC as an emerging CV

risk factor [18]. Research has established that non-HDLC, sdLDLC, and the HDLC/LDLC ratio as individual tests are in some cases superior to LDLC or HDLC alone in the atherosclerotic associated cardiovascular (CV) risk diagnosis and lipid lowering drug treatment monitoring [1,15,16,17,18]. The incorporation of sdLDLC into dyslipidaemia diagnosis and treatment targets is expected in the upcoming revised NCEP ATP edition [19].

Routinely, laboratories in Namibia and most parts of the world make use of the Friedewald method in calculating the LDLC levels from the measurements of TC, TG, and HDLC. Although convenient, the Friedewald method suffers from well-established limitations, which led the NCEP Expert Panel to recommend the development of accurate direct homogeneous methods. The Friedewald method limitations include a total analytical error of >12%, which is unacceptably high according to the NCEP guidelines [2,11,14].

The NCEP, through the Cholesterol Reference Laboratory Method Network (CRLMN) recommends that manufacturers develop direct homogeneous reagent methods which must be evaluated and accepted by the CRLMN laboratories [20]. In addition to that, the methods must pass validation by routine clinical laboratories and only then can the methods be adopted. It is unclear how the available direct homogeneous methods compare with the Friedewald method and the beta-quantification (BQ) reference method. Limited evaluations of the direct homogeneous assays raise questions about their reliability and specificity, especially in samples with atypical lipoproteins [14]. Also, some of the studies were conducted in specialised lipid laboratories and may not be representative of performance in routine clinical laboratories [14,21].

This study investigated the CV risk prediction of cLDLC, dLDLC, non-HDLC, sdLDLC, and the HDLC/LDLC lipid ratios from routine fasting and non-fasting lipid profile specimens received at selected private laboratories in Windhoek, Namibia.

There is a growing interest in identifying and quantifying other biomarkers, other than the routine lipid biomarkers (such as LDLC) as predictors of CVD. Standard lipid profiling fails to identify CVD risk in a substantial portion of the population [10,22]. The standard lipid fraction measurements, including LDLC should be complimented with other tests such as the non-routine lipid calculations like non-HDLC, sdLDLC, and lipid ratios; and non-lipid analyses like inflammatory cytokines. Selecting the best CV risk biomarker profile from an expanded list of CV risk biomarkers will improve the diagnosis, prognosis and understanding of clinical and premature atherosclerosis pathogenesis [10]. The inclusion of other biomarkers will enhance the accuracy of atherosclerosis risk prediction, enhance the accuracy of outcome prediction, and assist in treatment selection and adjustment in patients with atherosclerosis or CVD [10,19,22].

1.2 Statement of the problem

Due to the high total analytical error(>12%) of the commonly used Friedewald method in diagnosing hypercholesterolemia (elevated LDLC) in patients and the narrow CV risk categories, there is a high likelihood that some patients are being classified into incorrect risk categories of developing CVD, and subsequently incorrectly treated. Direct assays with acceptable analytical error (<12%), such as the Roche Diagnostics direct homogeneous method, are an alternative to the Friedewald method. However these assays have not been

universally adopted in laboratories in Namibia and most parts of the world due to lack of correlation studies and apparent cost constraints. The use of other lipid calculations like non-HDLC, sdLDLC, and lipid ratios in lipid profiling will give more information about the dyslipidaemias without increasing the cost of laboratory analysis. The routine lipid profiling misses some subclinical cases at the risk of development of CVD. These subclinical cases might be diagnosed using other biomarkers.

1.3 Objectives

a) To compare the Roche Diagnostics direct homogenous LDLC method (dLDLC) and the Friedewald LDLC calculation method (cLDLC).
b) To compare the classification of sera into CV risk categories using the cLDLC, dLDLC, non-HDLC, HDLC/LDLC, and the sdLDLC.

1.4 Significance of the study

A comparison of the Friedewald method and the Roche Diagnostics direct homogenous method will establish the better method for measuring LDLC in serum. In addition, the study will also establish the significance and usefulness of some non-routine lipid calculations in prediction of CVD. The research findings will contribute towards improving the diagnosis and management of patients who are prone to the development of AACVD and those with clinical AACVD. Currently, no studies have been conducted in Namibia to improve on the diagnosis and management of CVD through the routine lipid measurements and calculations, and the non-routine lipid calculations like the lipid ratio, non-HDLC, and the sdLDLC.

2. LITERATURE REVIEW

2.1 Cholesterol metabolism

2.1.1 Nature of cholesterol

Cholesterol belongs to the sterols group of biochemical substances which also includes other molecules like bile acids. The sterols together with fatty acids (FAs) and fatty acid derivatives such as TGs and phospholipids, steroid hormones, and the fat soluble vitamins (A, D, E, K) belong to a heterogeneous group of biological molecules making up lipids [6,15,23].

Cholesterol is the major sterol in human beings and every living organism contains sterols. It is found almost exclusively in humans and other animals where it is present in all body cells and most body fluids [11,23]. Lipids have important roles in virtually all biological life aspects with cholesterol being the starting point in many metabolic pathways [15]. The majority of the body cholesterol is in the free un-esterified form which is the structural component in hormones and in cell membranes where it maintains cell fluidity and permeability [6,24]. Cholesterol forms an insulation layer around nerve cells and is required in the production of vitamin D and bile acids in the body [15,24]. Furthermore, cholesterol metabolism results in generation of fatty acids (metabolic fuels) and TGs (energy storage) [11,15,24].

Sterols other than cholesterol are termed non-cholesterol sterols. Sitosterol, campesterol, and stigmasterol are examples of non-cholesterol sterols [11,26]. Most non-cholesterol sterols are of plant origin and are called phytosterols. Some few non-cholesterol sterols are of animal origin [19,24,25]. The phytosterols compete with cholesterol for absorption from the gut [11].

2.1.2 Lipoproteins

Lipids are chemically diverse substances which are soluble in organic solvents and nearly water insoluble [6]. Lipids are hydrophobic. Lipids such as cholesterol and TGs need to be transported to the distant tissues and organs in order to exert their metabolic functions. The lipids are transported in plasma in protein-coated hydrophilic vehicles/carriers called lipoproteins [6,15]. Lipoproteins are a diverse group of sub-microscopic and macromolecular spherical or discoid complexes composed of cholesterol, TGs, phospholipids, and apoproteins [22]. All lipoproteins share a similar structure consisting of an outer monolayer of the amphipathic protein which includes apolipoproteins/apoproteins and enzymes; and polar lipids (phospholipids and un-esterified cholesterol) surrounding an inner hydrophobic core of neutral lipids namely TGs and cholesterol esters held by non-covalent forces (Figure 1) [23,25,27]. The hydrophilic surface protects the hydrophobic core from the aqueous body environment [25]. The lipoproteins solve the problem of lipid/water incompatibility of lipids by exploiting the amphipathic nature of phospholipids and apolipoproteins. One end of the phospholipid molecules is polar and the other end is non-polar. Lipoproteins differ in their relative concentrations of protein to lipid and in their constituent lipids and proteins [6,11,23,25].

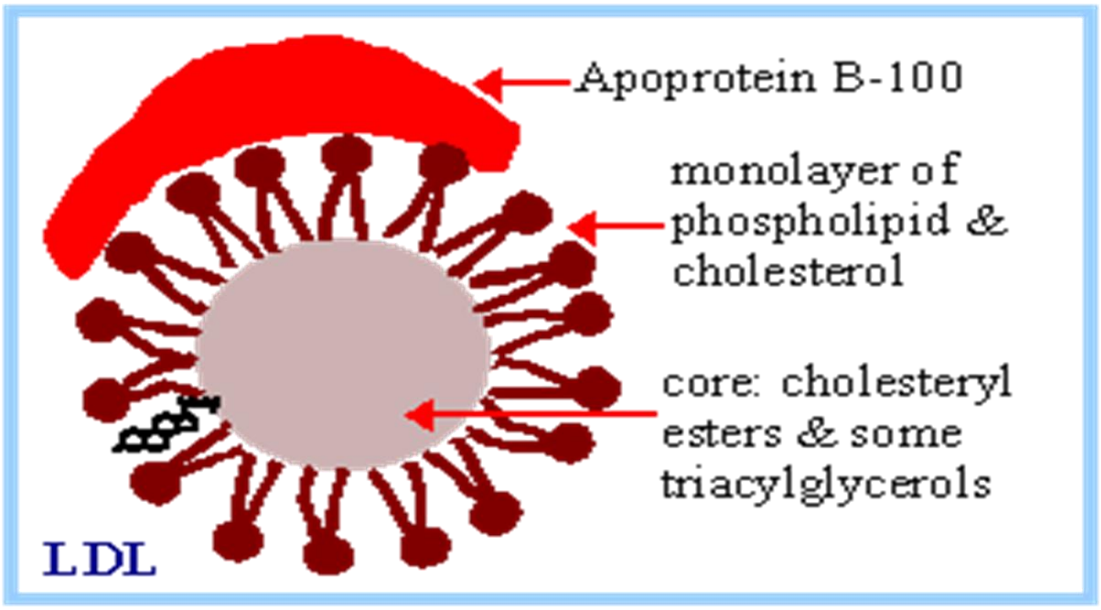

Figure 1: Low Density Lipoprotein (LDL) structure [27]

2.1.3 Apolipoproteins

Apolipoproteins are a very heterogeneous protein family which forms the protein component of the lipoproteins. The apolipoproteins determine the metabolism of lipoproteins by conveying solubility to the lipoproteins, directing lipoprotein transport and redistribution between tissues, functioning as co-factors for a variety of enzymes, and by contributing to plasma lipoprotein structural stabilisation [24,25]. The understanding of apolipoproteins contributes to the understanding of atherogenesis and the development of new treatment strategies in dyslipidaemia [8,26].

2.1.4 Sources of cholesterol

The human body cholesterol has three sources namely dietary, de novo, and recycled/extrahepatic cholesterol. The de novo and recycled/extrahepatic cholesterol sources are the endogenous sources of cholesterol and the dietary cholesterol is the exogenous source of cholesterol [6,25]. The maintenance and regulation of cholesterol prevents cholesterol build up in the body. The cholesterol build up is responsible for pathological conditions like atherosclerosis and gall stone formation [24]. The body cholesterol levels are affected by de

novo synthesis, dietary absorption, metabolism of cholesterol to bile acids and sterol hormones, and excretion and reabsorption as bile acids [9,23]. The cholesterol homeostasis in the cell is achieved by the regulation of the cholesterol synthesis rate determining reductase enzyme, regulation of the LDL receptor synthesis, and regulation of the esterification and removal of the free cholesterol [24].

2.1.4.1 De-novo synthesis of cholesterol

Most tissue and plasma cholesterol is synthesised endogenously by the liver and other tissues from simpler molecules particularly acetate [6,11]. In the initial stage of cholesterol biosynthesis, acetyl CoA moieties derived from carbohydrates, amino acids, and FAs are condensed to form acetate, which is the building block of cholesterol [24]. Hepatocytes are the source and often the acceptors of particles involved in the endogenous cholesterol pathway [25]. All the body cells have the capacity to synthesise cholesterol from acetate but about 90% of the cholesterol synthesis occurs in the liver and the intestine with the majority of the cholesterol synthesis occurring in the liver [6,11]. Peripheral cells and other organs largely get their cholesterol from the blood circulatory system [24]. The dietary lipids and endogenously synthesised lipids are transported by blood in the lipoproteins. The hepatic synthesis of cholesterol is inhibited by newly absorbed cholesterol, which reaches the hepatocytes in the chylomicron remnants. The excess newly absorbed or synthesised cytoplasmic cholesterol inhibits a reductase enzyme activity, which is limiting in cholesterol biosynthesis. This negative feedback mechanism assists in the control of the cholesterol body pool by adjusting the rate of endogenous synthesis against the rate of dietary absorption [11,25]. The cholesterol synthesis rate determining reductase enzyme is regulated by some transcription factors known as sterol regulatory element binding proteins (SREBPs) as well as the phosphorylation of the reductase enzyme. The SREBPs are attached to the endoplasmic

reticulum and the nucleus membrane surfaces. In decreased cytosolic cholesterol concentrations, the SREBPs are released and enter the nucleus. Once inside the nucleus, the SREBPs attach to sterol regulatory elements, initiating the transcription of LDL receptors and the cholesterol synthesis determining reductase enzyme. The increased transcription of the LDL receptors and the reductase enzyme increase the intracellular cholesterol levels [24].

The hepatic derived TGs and cholesterol combine with phospholipids and apo B100 to form the TG - rich VLDL particles. The liver secretes the de-novo synthesised TG-rich VLDLs [25]. The VLDL is hydrolysed by lipoprotein lipase to give cholesterol esters and apo E rich remnant particles (a process similar to chylomicron hydrolysis). Approximately 50% of the VLDL remnants are cleared by the liver in a process that involves uptake by the LDL receptor related protein, which recognises apo E. Approximately 50% of the remainder VLDL remnants undergo further hydrolysis by hepatic lipase to form IDL which is hydrolysed to LDL. LDL is the major cholesterol carrying lipoprotein in the plasma accounting for at least 70% of the plasma cholesterol. The only protein contained in the LDL particle is a single molecule of apo B-100 [11,25].

LDL protein receptors are present on hepatocytes and also on peripheral tissue cells. Approximately 50% of plasma LDL uptake by the LDL receptor mediated mechanism is hepatic. The major determinant of plasma LDL concentration is the number of functional LDL receptors. In subjects who lack functional receptors for example in homozygous familial hypercholesterolemia, LDL concentrations are markedly elevated. In this case, LDL removal from the blood is entirely dependent on the less efficient non-LDL receptor mediated mechanisms. LDL receptors recognise both apo B-100 on LDL and apo E on remnant

particles and HDL. Once a lipoprotein is bound to the receptor, the receptor – lipoprotein complex localises in the coated pit region from where it is internalised by endocytosis. Upon entering the cell, cholesterol esters are hydrolysed by esterases. The lack or malfunction of the esterases results in cholesterol ester storage disease. The LDL receptor is recycled while the lipoprotein undergoes lysosomal degradation to un-esterified cholesterol and amino acids [11].

2.1.4.2 Cholesterol Absorption

Cholesterol is presented to the intestinal wall from three sources namely the diet, the biliary secretions, and the intestinal secretions [6,11,25]. The bulk of the dietary cholesterol comes from animal products, for example meat, egg yolk, sea food, and whole fat dairy products [6,11]. All the intestinal cholesterol (dietary, biliary, and cellular) is presented to the small intestine mucosal cells (enterocytes) in the form of un-esterified (free) form. Esterified cholesterol in the diet is first rapidly hydrolysed in the intestine to free cholesterol and free FAs by cholesterol esterases in the pancreatic and the small intestinal secretions [11]. The enterocytes absorb dietary cholesterol and TGs from the gut in the form of free cholesterol, FAs, and monoglycerides. In order for lipids to be absorbed into the circulatory system from the enterocytes, the free un-esterified cholesterol must first be solubilised. The solubility is achieved by the formation of chylomicrons which contain un-esterified cholesterol, FAs, monoglycerides, phospholipids, and conjugated bile acids [24]. Bile acids have amphipathic properties which allow chylomicron formation. The chylomicron formation is a crucial step in cholesterol absorption. In the absence of bile acids, the digestion and absorption of cholesterol and TGs are severely impaired [11,25].

After its absorption into the enterocytes, cholesterol is first re-esterified to form cholesterol esters [23]. Cholesterol esterification is a process whereby a fatty acid is combined with the cholesterol at its one vaguely polar spot to make an even more non-polar molecule. The cholesterol esters are reassembled along with TGs, phospholipids, and a number of apolipoproteins into chylomicrons. These esters and TGs form the core of the chylomicron particles. The enterocytes synthesise apo B-48 (the major protein of chylomicrons) and other apolipoproteins. All the apolipoproteins together with the phospholipids form the surface layer of chylomicrons [11,23,25].

Apo B-48 is vital in the formation and secretion of chylomicrons. There is only one apo B-48 molecule per chylomicron particle which remains with the chylomicron particle throughout the chylomicron life until the chylomicron remnant it is taken up by the liver [23]. In contrast, multiple copies of the other apolipoproteins are present in a single chylomicron and the apolipoproteins are exchanged with other lipoproteins throughout the chylomicron's life. For example the chylomicron apo A is transferred to high density lipoprotein particles (HDLPs) [23,25].

The enterocytes release the chylomicrons into the lymphatics which empty into the thoracic duct before they eventually enter the systemic venous circulation [25]. Lipoprotein lipase acts extracellularly to hydrolyse the TGs within the chylomicron cores to give FAs. The FAs are either utilised as an energy source or re-esterified and stored in adipose tissue as TGs. The hydrolysis of chylomicrons also generates and releases into circulation chylomicron remnant particles which have a relatively high content of cholesterol esters and apo E. Normally chylomicrons are quickly cleared from the plasma by the liver. In persons with a rare

deficiency of apo B-48 synthesis, chylomicron formation and consequently fat and cholesterol absorption are severely impaired [11].

Thirty to sixty percent of dietary and extrahepatic cholesterol is absorbed daily. In contrast, only less than one percent of the non-cholesterol sterols are absorbed. There is wide inter-individual variation of the quantities of absorbed cholesterol [11,25]. Cholesterol absorption is affected by the quantity of dietary cholesterol. As dietary cholesterol concentration increases, the quantity of absorbed cholesterol increases to a maximum of approximately 1g/day when the oral intake reaches a maximum 3g/day. The cholesterol absorption varies considerably according to the dietary intake of animal products. A normal Western diet contains approximately 200 – 500mg/day of animal cholesterol [25]. Also, approximately 400 – 700mg/day of cholesterol comes from the biliary secretion and the turnover of mucosal cells [11]. In addition to animal cholesterol, approximately 200 – 400mg/day of non-cholesterol sterols (mainly plant sterols but also some fish sterols) are ingested daily. Plant sterols are poorly absorbed and when they are administered in amounts of 5 – 15g/day, they significantly inhibit the absorption of cholesterol [9,25].

2.1.5 Cholesterol excretion

The cholesterol reaching the liver is either secreted unchanged into bile or metabolised to bile acids. A significant amount of cholesterol is always excreted directly into the biliary system where it is normally solubilised by mixed micelles of bile acids and phospholipids [11,24]. If the amount of cholesterol exceeds the capacity of the solubilising agents, a supersaturated state occurs forming lithogeny bile. This excess cholesterol can precipitate and result in the development of cholesterol gallstones which account for approximately 80% of all gallstones in Westernised societies [11].

Approximately one third of the daily production of the hepatic cholesterol is first esterified, and then catabolised in the liver into two primary bile acids namely cholic acid and chenodeoxycholic acid. Cholic acid and chenodeoxycholic acids are conjugated with either glycine or taurine and enter the bile canaliculi. In the small intestine, the conjugated bile acids are involved in cholesterol and fat absorption [6,24]. Some of the bile acids are de-conjugated and converted by bacteria in the intestine to secondary bile acids. Cholic acid is converted to deoxycholic acid while chenodeoxycholic acid is converted to lithocholic acid. Virtually all bile acids except lithocholic acid are reabsorbed in the small intestine and returned to the liver, thus completing the enterohepatic circulation [6,11]. Ninety seven percent of the bile acids are reabsorbed in the small intestine and three percent of the bile acids are excreted. The three percent excreted bile acids represent one of the exits of cholesterol from the body. The other exit of cholesterol from the body is the loss of dead cells from the epidermis. The loss of cholesterol through excretion and exfoliation are important in cholesterol homeostasis [24].

2.2 Classification of Lipoproteins

The varied physical and chemical properties of lipoproteins allow for an operational definition and the subsequent classification of the lipoproteins based on their varying protein and lipid composition, size, density, electrophoretic mobility, and function [6,11,22]. Lipoproteins have been classified, quantified and eventually correlated with the various disease states by using various separation techniques such as ultracentrifugation, nuclear magnetic resonance (NMR), electrophoresis, chromatography, and vertical auto profile (VAP) [6,22,26]. The nomenclature of the lipoproteins is routinely based on the density of the lipoprotein particles when separated by ultracentrifugation. This density classification has divided lipoproteins into five main density classes namely chylomicrons (<0.95g/mL), VLDL

(0.95 – 1.006g/mL), intermediate density lipoproteins (IDL) (1.006 – 1.019g/mL), LDL (1.019 – 1.063g/mL), and HDL (1.063 – 1.210g/mL) [11,22]. The density of lipoproteins is inversely related to their size. Lipoprotein classes are not homogeneous. Each lipoprotein represents a continuum of particles of differing size, density, fate, and in the case of VLDL, also of origin. In some instances, in spite of contamination with adjacent lipoprotein fractions, ultracentrifugation remains the golden standard method for lipoprotein research and diagnostics [18,22,23].

Lipoproteins are classified into alpha (α), pre-beta (β), and beta (β) lipoproteins which are HDL, VLDL, and LDL classes respectively according to their electrophoretic mobility. The separation of different lipoproteins into sub-classes is done using chromatographic methods based on the size of lipoprotein particles or their immune-affinity. The high resolution nuclear magnetic resonance (NMR) spectroscopy technique is used to separate lipoprotein subclasses according to their different sizes. Some small LDL particle phenotypes are more atherogenic than others. Therefore the separation of the lipoprotein subclasses and the estimation of their proportion in a particle population improve the risk estimation of CVD [23,26].

Associated with the five density classified lipoprotein classes are at least five major apolipoproteins namely apo A, apo B, apo C, apo D and apo E [22]. All the major apolipoprotein groups except apo D have subgroups. The major apolipoprotein groupings and their subgroups differ in their primary, secondary, and tertiary structures, in their physiochemical behaviour, and their function and distribution in the various lipoprotein species [11,25].

Apo A is found in both HDL and chylomicrons. Apo B has two subgroups, namely apo B-100 and apo B-48. Apo B-100 is found in the lipoproteins that are synthesised from the liver which include VLDL, VLDL remnants, IDL, and LDL. The apo B-48 is synthesised in the intestinal wall and is found in chylomicrons [22]. Apo B is found on atherogenic lipoproteins. All lipoproteins except HDL contain apolipoprotein B in the form of either apolipoprotein B-48 or apolipoprotein B-100 which makes it possible to assess the total amount of atherogenic lipoprotein particles in the circulation by the apolipoprotein B measurement. In normal serum samples, very little if any apo B-48 is found in fasting plasma. Also in fasting normal serum samples, the apo B-100 is almost exclusively from the LDL [11].

The synthesis of apo B and its incorporation into chylomicron and VLDL are essential for the formation and release of these lipoproteins into the plasma. Hepatic synthesised apo B leaves the liver on VLDL. The VLDL is gradually converted to the relatively TG-poor LDL molecule and the apo B assumes a major role in ensuring lipid delivery to the extrahepatic cells [22]. The apo B assumes the role of a recognition protein on the surface of the lipoprotein, allowing the recognition and binding of LDL to specific high affinity receptors on the cell membrane [11,22]. With the exception of the nervous system and red blood cells, all tissues have receptors for apo B in the LDL form [11].

The catabolism of apo B occurs mainly via a receptor mediated pathway. Once internalised in the cell, apo B is degraded to its constituent amino acids by lysosomal enzymes with apo B being catabolised by non-receptor mediated processes that include a scavenger pathway [11].

Table 1: Classification properties and composition of human serum lipoproteins [6,11,22]

Parameter	Lipoprotein					
	Chylomicron	VLDL	IDL	LDL	HDL	sdLDLC
Density (kg/L)	<0.94	0.94-1.006	1.006-1.019	1.019-1.063	1.121	1.050-1.063
Size/Diameter (nm)	75-1 000	30-80	25-40	23-35	7.5-10	22-25.5
Unesterified chol (%)	2	5 - 8	8	13	6	ND
Esterified chol (%)	5	11 - 14	22	39	13	ND
Phospholipid (%)	7	20 - 23	25	17	28	ND
TG (%)	84	44 - 60	30	11	3	ND
Protein	2	4 - 11	15	20	50	ND
apoB-100 (% total apo)	trace	36.9	50 - 70	98	trace	ND
apoB-48 (% total apo)	22.5	Trace	Trace	Nil	Nil	Nil
apo	B48, CI,CII, CIII,E	B100,CI, CII,CIII,E	B100,CI,CII, CIII	B100	AI,AII,CII, CIII,E	B100
synthesis	intestine	liver	intravascular	intravascular	liver	intravascular

ND – classification not done

2.2.1 Chylomicrons

Lipids from the diet and extrahepatic circulation are transported to the intestinal wall and integrated into chylomicrons. The chylomicrons are the largest and most buoyant class of lipoprotein which are rich in TGs [15]. Chylomicrons are the lipoproteins responsible for transporting dietary lipids. They are synthesised by and released from the intestinal epithelial cells [24]. The lipid content is derived predominantly from the alimentary tract and is considered to be exogenous. The major lipid fraction is TGs which constitutes more than 80% of the total particle by weight. Chylomicrons contain various apolipoproteins, but only 1 – 2% of the total particle is made up of apolipoproteins. The most notable apolipoproteins on the chylomicrons are apo B 48, apo C, and apo E with the major protein component being apo B 48. The chylomicrons enter the lymphatics and finally the systemic circulation and while travelling through the lymphatic system, the chylomicrons acquire significant

quantities of the apo C from HDL particles [11]. Unlike many other serum components that are mostly transferred from one point to another as metabolites, lipoproteins undergo a series of complex metabolic processes in which changes and exchanges occur continuously in and between the various lipoproteins, a process referred to as lipoprotein cascade. In the systemic circulation, chylomicrons are hydrolysed by lipoprotein lipases, degrading the chylomicron TGs by TG lipolysis into free fatty acids, glycerol, IDL, and chylomicron remnants [6,11,23]. Apo B-48 and apo E are the main apolipoproteins of the chylomicron remnant. The remnant particle retains most of the cholesterol esters found in the chylomicron and are rapidly removed from the circulatory system by the liver, usually in less than 10 minutes. All lipoproteins are catabolized in the liver, kidney, or peripheral tissues via apolipoprotein receptor interactions or other receptor independent mechanisms [11,15,23,25].

Chylomicron remnants are taken up by the liver via LDL receptor pathway or LDL receptor related protein. Both pathways use the chylomicron remnant particle apo E as a ligand with the process being mediated by the liver apo E receptor. The remnants are rapidly internalised by the receptor mediated endocytosis and degraded in the hepatic lysosomes, thus delivering dietary cholesterol to the liver. The IDL is in turn hydrolysed by hepatic lipase and then converted into LDL [15]. The IDL and the LDL transport cholesterol, TGs and other lipids to peripheral tissues in a process called forward cholesterol transport [15,23].

2.2.2 Very low density lipoprotein (VLDL)

The metabolism of VLDL is identical to that of chylomicrons with the principal difference being the source of the lipids [24,25]. VLDL is a TG enriched lipoprotein which is synthesised in and released from the liver [15]. The core of VLDL is predominantly composed of TGs and it is the largest of the lipoproteins containing endogenously produced

lipids. VLDL transports hepatic synthesised TG and cholesterol which are derived from dietary precursors such as free FAs, glycerol and carbohydrates. VLDL synthesis is stimulated by energy excess without regard to calorie source. Besides TG, VLDL contains about 10% cholesterol and a number of functionally important apolipoproteins. The major protein is apo B-100 which is identical to the LDL apo B although their behaviour is significantly different. The VLDL apo B-100 is of high molecular weight and does not significantly bind to the apo B (LDL or apo E) receptor. The apo C is vital to VLDL catabolism and is transferred to HDL together with some lipid material during the catabolic process. VLDL also undergoes the process of lipoprotein cascade.The apo C on HDL is later transferred back to chylomicrons and VLDL to complete the cycle. The half-life of VLDL in serum is 1 -3 hours [11,23,25].

The extracellular lipoprotein lipase degrades VLDL into free FAs, IDL and VLDL remnants. When VLDL decreases their TGs concentration due to LPL hydrolysis, they are converted into IDL and VLDL remnants. The remnant particles are rapidly removed from the circulatory system by the liver. The IDL is in turn hydrolysed by hepatic lipase and then converted into LDL. The IDL, LDL, and VLDL transport cholesterol, TGs, and other lipids to peripheral tissues in a FCT process [15,23].

2.2.3 Intermediate density lipoprotein (IDL)

These particles are produced during the conversion of chylomicrons and VLDL to LDL. The chylomicrons and VLDL are hydrolysed by lipoprotein lipase to form a short-lived IDL, which is partly depleted of TGs. The IDL is converted to LDL [11,24]. IDL core contains almost equal amounts of cholesterol esters and TGs but its density lies between those of

VLDL and LDL. The major apolipoproteins of IDL are apo B and apo E with apo E determining the continued catabolic process of IDL conversion to LDL [15,23].

2.2.4 Low density lipoprotein (LDL)

LDL is the major cholesterol - containing lipoprotein with its core comprising mainly of cholesterol esters and apo B-100 being the main protein component [26]. LDL represents the end – product of chylomicron and VLDL metabolism. Extracellular lipoprotein lipase degrades TGs of chylomicrons and VLDL into free fatty acids, IDL and remnants. When chylomicrons and VLDL decrease their TGs concentration, they are converted into IDL and remnants while IDL is further hydrolysed by hepatic lipoprotein lipase to form LDL [15]. The IDL, LDL, VLDL, and VLDL remnants transport cholesterol to peripheral tissues. LDL is the most cholesterol enriched lipoprotein particle in the blood circulation, transporting about 70% of plasma total cholesterol [14]. It is responsible for constant supply of cholesterol for tissues and cells [11,15]. The LDL particles are crucial for maintaining cholesterol homeostasis. The persistence of elevated LDL particles is directly linked to atherosclerosis [24].

LDL is a heterogeneous class of lipoproteins consisting of a set of discrete subspecies with distinct properties including size and density [18,22,28]. The LDL population is sub-divided according to size and density into large buoyant LDL (lbLDL) variant and small dense LDL (sdLDL) variant particles [15,18]. The prevalence of small particles varies with genetic factors, diet, age, and gender. Elevated levels of small LDL particles are observed in 30 – 35% of men of at least 20 years of age, 5 – 10% in men under 20 years and premenopausal women, and in 15 – 25% in post-menopausal women. Elevated sdLDLC particles are also associated with metabolic syndrome (metS) and lipid lowering drug therapy (LLDT) [18,23].

LDLC is removed from the plasma primarily by liver parenchymal cells. The LDL receptors are more concentrated on the liver parenchymal cells than the peripheral body cells [11]. The liver LDLC receptors are responsible for regulating the cholesterol pool of the body plasma. The LDLC is also removed by the body peripheral cells. The primary function of the peripheral cell LDL receptors is to enable the peripheral cells to get the plasma pool cholesterol [24]. In both the hepatic and peripheral body cell types, the LDLC is internalised into the cells using either the high affinity specific apo B LDLC receptors located in the coated pits or using a non-specific receptor independent scavenger mechanism. The majority of the LDL is internalised by the high affinity receptor pathway at the coated pits and the remainder is removed by the scavenger cell system [15,24]. In the LDL high affinity receptor mechanism, the LDL interacts with high affinity receptor sites located in regions of the cell membrane called coated pits. The bound LDL is then internalised by invagination of these pits into the cell, where the pits pinch off to form endocytic vesicles. The rest of the LDLC is internalised through a receptor independent scavenger mechanism [11]. Coated pits account for about 2% of the cell surface area. However the coated pits account for about 50 to 80% of the LDL receptors [24].With increasing plasma levels of LDL, the scavenger cells non-specifically take up larger amounts of the circulating lipoprotein for degradation. After the LDL uptake using either of the two internalisation methods, the endocytic vesicles fuse with intracellular lysosomes where the LDL moiety is subjected to hydrolytic enzymatic degradation that hydrolyses the apo B to amino acids. The esterified cholesterol in LDL is hydrolysed by lysosomal cholesterol esterase releasing the free cholesterol into the cytoplasm [11,26].

The release of free cholesterol is responsible for two regulatory responses that assist in cholesterol homeostasis through feedback mechanisms. Firstly, excess cytoplasmic free

cholesterol suppresses the rate limiting, reductase enzyme and therefore suppresses new cholesterol synthesis [11,24]. Excess cytoplasmic free cholesterol, synthesised or entering the hepatocytes is detoxified by esterification and storage intracellularly in the form of cytosolic cholesterol ester droplets [11]. Secondly, excess free cholesterol reduces the number of LDL receptors on the target cell plasma membrane preventing the subsequent over-accumulation of intracellular cholesterol through the receptor pathway. The number of the high affinity LDL receptors on the target cell plasma membranes reflects the activity of those cells with respect to cholesterol dependent anabolic reactions. Although high affinity LDL receptors are found on most cells, they are numerous in certain cell types such as the adrenocortical cells, where LDL-derived cholesterol provides the major source of substrate for synthesis of steroid hormones [11,26].

The non-specific pathways for example the bulk endocytosis are not subject to feedback regulation. The LDL non-specific degradation modes are less efficient and require elevated plasma levels to achieve significant rates of removal. The bulk endocytosis occurs in the scavenger cells or macrophages of the reticuloendothelial system. With increasing plasma levels of LDL, the scavenger cells take up larger amounts of the circulating lipoprotein for degradation. Macrophages bind and internalise oxidised forms of LDL. Oxidative modification of LDL can occur by a number of mechanisms, accelerating the potential for atherosclerosis. At some point the macrophages become overloaded with cholesterol esters and take on the appearance of foam cells. In the arterial wall, both macrophages and smooth muscles accumulate cholesterol esters by this mechanism. Foam cells are the hallmark of atherosclerotic plaque [11].

Patients with the genetic disorder familial hypercholesterolemia have a defect in the gene coding for LDL receptors. The defects of familial hypercholesterolemia are the homozygous state (the functional receptors are usually absent or may be very few), the heterozygous state (the heterozygous persons have approximately half the normal number of LDL receptors), and the defective receptors (in some cases the receptor is present and bind normally but the receptor is unable to internalise the LDL particle). The inability of the LDL moiety to deliver cholesterol to the cells of patients with familial hypercholesterolemia results in increased cholesterol synthesis. Also, the reduction in the removal of LDL from plasma causes elevation in its plasma levels, increased uptake by macrophages and smooth muscle cells, cholesterol deposits in arterial walls, and consequently premature atherosclerosis [11].

2.2.5 High Density Lipoprotein (HDL)

HDL is the smallest and densest of all the lipoproteins. Seventy percent of the HDL is secreted from the liver and 30% is secreted from the intestine. There is an inverse relationship between the levels of HDLC and CVD [20,21]. HDL transports excess cholesterol from the peripheral cells or the cholesterol deposited in the arterial wall back to the liver in a process called reverse cholesterol transport [29]. The cholesterol is excreted in bile [15,23].

There are several athero-protective processes involved in reverse cholesterol transport. These include cholesterol efflux from peripheral cells, esterification of cholesterol in HDL, cholesterol transfer from atherogenic lipoproteins to HDL, and remodelling of HDL and uptake of HDLC by the liver [6]. HDL has other anti-atherogenic properties which include down regulation of the expression of adhesion molecules on the surface of the vascular endothelium, inhibition of platelet aggregation, stimulation of endothelic nitric oxide production and anti-oxidant activation, upregulation of ATP binding cassette protein A1, and

activation of intracellular signalling leading to stimulation of cholesterol efflux [28]. Increased HDL retards or prevents the development of atherosclerosis [23,25].

2.3 Atherosclerosis

In the physiological state, LDL and to a lesser extent IDL and remnants transport cholesterol from the liver to the peripheral body cells for storage in a forward cholesterol transport process [29]. HDL enters the artery wall where it prevents or reverses the process of atherosclerosis by inhibiting and reversing the oxidation of LDL particles and removing cholesterol from foam cells for delivery to the liver [22]. Under normal conditions, excess cholesterol is removed from the sub-endothelial space via this reverse cholesterol transport [6,22].

Serum lipoprotein disturbance is an etiological factor in the development of atherosclerosis [26]. The lipoprotein disturbance increases the likelihood of the altered or unaltered lipoprotein function and/or level entering the arterial intima and participating in foam cell formation. This by itself or through interaction with other CV risk factors result in atherosclerosis [1]. Lipid metabolism can be disturbed in various ways which include alteration in plasma lipoprotein concentration, plasma lipoprotein particle size, plasma lipoprotein biochemical nature, and plasma lipoprotein function [1,26].

Lipoprotein particle concentration is the major factor determining whether a lipoprotein will invade the arterial intima following the vascular endothelium dysfunction. The atherogenic particle concentration is the most significant factor in atherosclerosis and subsequent CVD

clinical events. The atherogenic cholesterol particles are driven into the arterial intima by concentration gradients [26].

The main atherogenic lipoprotein particles, namely LDLP, IDLP, VLDLP, chylomicron particles, and remnant particles are dependent on either hepatic or extrahepatic receptors for their ultimate metabolism [25]. The chylomicron remnant and IDL are taken up via apo E hepatic receptors, permitting delivery of large amounts of dietary cholesterol to the liver, which in turn modulates endogenous cholesterol synthesis. In dysbetalipoproteinemia, the person lacks apo E. This disrupts the remnant cholesterol and IDL liver clearance. This causes serum cholesterol levels to rise mainly due to dietary cholesterol. In these patients dietary restriction of cholesterol and saturated fat is often effective in lowering serum cholesterol levels [11].

Large amounts of endogenously synthesised cholesterol can be transported from the liver to the periphery by means of the LDL receptor mechanism without the need for high levels of LDL in plasma. However, this endogenous transport system can also be disrupted for example in familial hypercholesterolemia where the LDL receptor mechanism is defective. With a defect in the LDL receptor mechanism, circulating LDL and the plasma LDLC levels rise. Similarly in subjects with defective apo B, the LDL does not bind to the LDL receptor causing the plasma LDLC levels to rise. The defective apo B disorder is associated with premature atherosclerosis [11].

Therefore LDL and LDLC elevations are due to increased endogenous production, excessive dietary intake of lipids and calories, and defective receptors [6]. The dietary intake affects more on the exogenous cholesterol carrying lipoproteins, which are chylomicrons and the chylomicron remnants. Excess dietary lipids and calories stimulate the overproduction of VLDL in the liver. The subsequent catabolism of VLDL to LDL increases the LDLC which saturates the LDL receptor system. This predisposes one to atherosclerosis. Even in the individuals with minor genetic defects in the LDL receptor system, the ability to cope with excess LDLC production is controlled by minor genetic factors, environmental factors, and possibly hormonal factors that affect the number and activity of lipoprotein receptors. The interaction of genetic, environmental, and humoral factors in controlling the number of lipoprotein receptors partly explains the most common forms of hypercholesterolemia. These polygenic interactions also explain the significantly different within-populations responses to similar dietary constituents [11].

MetS and LLDT have changed the serum lipoprotein particle composition. The particle size of LDL in patients with metS or undergoing dyslipidaemia treatment has shifted towards small dense LDL as compared to the larger variant in healthy individuals. These changes have caused the predominance of the more atherogenic sdLDLC variant. These changes in the serum lipoprotein composition have challenged the traditional dyslipidaemia diagnostic tools. The proportion of sdLDL variant increases with increasing TGs levels and decreased levels of HDLC. This lipid combination is defined as atherogenic lipoprotein phenotype which is a typical feature in metabolic metS [23]. LDLC concentration can be normal or low while sdLDLC and TGs are high following dyslipidaemia treatment and also in metS conditions like obesity and type 2 diabetes resulting in the underestimation of CV risk in these conditions. SdLDL particles are excellent predictors of cardiovascular events especially

in connection with metS and in some cases following LLDT. Some researchers have reported that increasing sdLDLC concentration is a more powerful risk factor and therefore more powerful CV risk predictor than LDLC [23]. The sdLDL variant is three times more atherogenic than the large buoyant LDL variant. In most healthy people the major LDL subspecies is the large buoyant LDL [22]. The predominance of sdLDLC is a major component of an athoregenic lipoprotein variant and a source of increased CVD. The enhanced atherogenicity of sdLDL particles is attributable to several phenomena [13,18].

The sdLDL particles have lower affinity to normal LDL receptors than the normal LDLP; have a higher affinity for the intimal proteoglycans than the normal LDLP which increases their binding to proteoglycans in arterial intima [23]. This receptor-independent retention of sdLDL particles in sub-endothelial space overrides the normal LDL receptor mediated or the LDLC scavenger cholesterol metabolism regulation. This leads to accumulation of cholesterol mainly in the form of sdLDLC in extrahepatic tissue [18,28].

The sdLDL particles are more easily oxidised to oxLDL as compared to their larger variants, possibly due to increased content of polyunsaturated fatty acids and reduced quantities of antioxidants and free cholesterol on the sdLDLC [28]. Atherosclerosis is the end-result of the oxidative modification of LDL in the arterial wall by reactive oxygen species. The plasma contains several antioxidants which prevent the oxidation of lipoproteins. The oxidative modification of lipoproteins plays a central role in the pathogenesis of atherosclerosis. The oxidation of lipoproteins occurs in the blood vessel walls and is coordinated by the cells of the monocyte/macrophage system, endothelial cells, and smooth muscle cells [18,28]. The increased atherogenicity of LDL is associated with a predominance of sdLDL subpopulation

that is more prone to oxidative modification than the lbLDL. The oxLDLC is more atherogenic than the unaltered LDLC. Experiments show that the incubation of cells of the monocyte/macrophage system with native (un-oxidised) LDL does not result in foam cell formation [28]. Foam cell formation is a characteristic feature and starting point of atheromatous lesions.

The other etiological factors in the development of atherosclerosis include the lipoprotein interaction with other CV risk factors and endothelial function [26]. The apo B of the atherogenic particle (commonly sdLDLP), attaches to the arterial wall proteoglycans and the surface phospholipids are readily oxidised. Some of the oxidised phospholipids undergo an enzyme catalysed lipolysis to generate oxidised fatty acids and lysophosphatidyl choline. The oxidised fatty acids and lysophosphatidyl choline are proinflammatory and therefore create endothelial dysfunction and recruitment of macrophages through chemotaxis. Some of the modified or oxidised phospholipids and oxLDLC are internalised by the monocyte/macrophage scavenger receptors initiating foam cell formation. In some in-vitro experiments, after oxidation of the LDL and incubation with the cells of the monocyte/macrophage system, a rapid uptake of oxLDL occurred leading to cholesterol accumulation in the cells of the monocyte/macrophage system resulting in foam cell formation. Therefore, LDL oxidation is necessary in atherogenesis. OxLDL induce foam cell formation, and the oxidative modification of LDL is an important initial process that occurs prior to atherosclerosis in vivo [26,28].

The sdLDL particles have a prolonged plasma half-life. An increase in plasma time of LDLC and more so of sdLDLC increases the uptake of cholesterol into the artery intima [28].

The sdLDL more readily penetrates the arterial wall due to its small size. The LDL particle size in patients with metS or under dyslipidaemia treatment has shifted towards smaller as compared to the larger size in the healthy individuals. These changes have caused the pre-dominance of the more atherogenic sdLDLC variant in cases with metS or under dyslipidaemia treatment [18,28]. The large buoyant LDL variant is >25.5 nm while the sdLDL is ≤25.5 nm in diameter [28]. A particle larger than 70nm will not enter the artery wall. Very large VLDL sub-particles and chylomicrons (larger than 70nm) will not enter the arterial intima [26]. The VLDL, chylomicron remnants, IDL, and LDL particles easily enter the arterial intima if present in increased concentrations. Small and large LDL particles are the most atherogenic particles in patients because they are the most numerous atherogenic apo B particles present in plasma [18,23,26]

The sdLDL carries an additional number of LDL particles per cholesterol equivalent as compared to lbLDL particles. These factors enhance the atherogenicity of sdLDLC. [18,23].

Elevated LDLP and LDLC plasma concentration initiates an inflammatory process which results in formation of an atherosclerotic plaque [11,33]. The inflammatory process damages the endothelium. Atherosclerosis is both an atherogenic lipoprotein accumulation disorder and an inflammatory disorder [10,31,32,34]. The lipoproteins that carry cholesterol and TGs are the direct mediators of the atherosclerotic process and hence AACVD. The LDL particles and to a lesser degree the IDL, promote and accelerate atherosclerosis by entering the artery wall, their cholesterol becoming oxidised, and subsequently being ingested by the cells of the macrophage/monocyte system. This creates cholesterol rich foam cells. The foam cells are the building blocks of the atherosclerotic plaque. The chylomicron and VLDLP remnant lipoprotein particles are readily scavenged by macrophages without having to be oxidised like

LDLP and IDLP and become a major component of the plaque. The Lp(a) which are small dense LDL particles are easily oxidised by oxidising agents in the arterial intima of the vessel wall before being taken up by the cells of the monocyte/macrophage system to form foam cells [26,29].

Atherosclerosis involves more than just circulating lipoproteins and cellular enzyme systems such as lipoprotein lipase. Cellular and humoral systems (such as platelets, arterial smooth muscle, endothelium, macrophages, and cytokines), genetic, environmental, and local factors have significant roles in atherosclerosis [11,31,32,34]. The atherogenic lipoprotein accumulation, build-up of calcium and cellular debris within the intima of walls of large and medium size arteries, activation of inflammatory processes and the subsequent increase in the pro-inflammatory molecules (such as IL-1, IL-6, and TNF-α), and the endothelial dysfunction, initiate and accelerate plaque formation. Once the endothelial barrier is compromised, lipid flooding of the vascular wall, mobilisation of macrophages, expression of chemotactic factors, growth and proliferation of smooth muscle and connective tissue occurs [15]. See figure 2 for the process of atherosclerosis. Subsequently there is increased LDLC uptake by the monocyte/macrophage system as well as by smooth muscle cells in vessel walls. When the amount of atherogenic particles exceeds the reverse cholesterol transport capacity of HDL particles, cholesterol accumulates in monocyte derived macrophages leading to the formation of foam cells. The foam cell formation in turn leads to atherosclerosis and then AACVD. Atherosclerosis is a precursor of CVD. The process of atherosclerosis is associated with the hardening and narrowing of blood vessels. In advanced cases of atherosclerosis, the arteries are totally blocked or rupture. The atherosclerosis developments (namely hardening, narrowing, and rupture of vessels), lead to the clinical events of CVD. The CVD clinical events include coronary artery disease, peripheral artery

disease, chronic heart disease, and myocardial infarction [15,30,31,34,35]. Figure 2 below shows the process of atherosclerotic plaque formation.

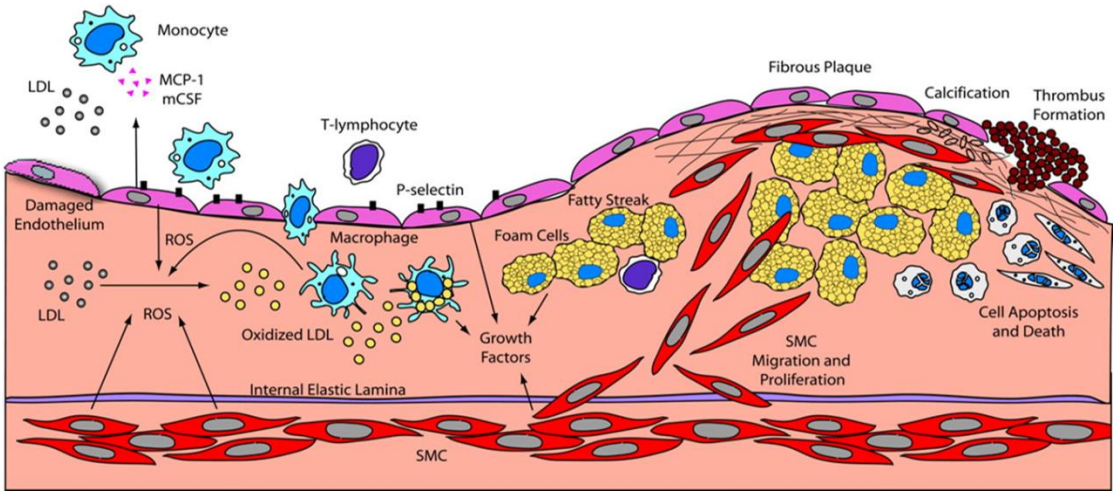

Figure 2: Atherosclerotic plaque formation in the arterial intima [36]

2.4 Cardiovascular Disease (CVD)

Atherosclerotic associated CVD is the commonest cause of CVD. The onset of CVD is consistently related to the presence of CV risk factors. The conventional CV risk factors are categorised into reversible, partially reversible, and irreversible [11,15]. The reversible risk factors include increased dietary cholesterol intake, sedentary lifestyle, obesity, arterial hypertension, alcoholism, stress, some disease states like hypothyroidism, some antiretroviral therapies and smoking. The partially reversible CV risk factors include diabetes and menopause while irreversible factors include family history of a CV risk factor/genetic factor for example inherited defects of lipid metabolism (such as familial hypercholesterolemia), male gender, and age. Therapeutic lifestyle intervention and drug therapy will cancel or reverse the reversible CV risk factor (s). Therapeutic lifestyle intervention and drug therapy will manage better the effects of the partially reversible and irreversible CV risk factors [15].

Most of the CV risk factors in the three risk classes of reversible, partially reversible and irreversible result in the development of the dyslipidaemia [6]. Environmental factors such as increased dietary intake and sedentary lifestyle or lack of exercise, inherited defects of lipid metabolism such as familial hypercholesterolemia, some diseases like diabetes, some therapies like antiretroviral treatment (secondary hypercholesterolemia) or any combination of these factors cause dyslipidaemia [1,6,11]. Most of the AACVD is caused by poor diet and a sedentary lifestyle. The genetic dyslipidaemias such as familial hypercholesterolemia and familial hypertriglyceridemia are less common but more severe [10,11,23]. The dyslipidaemia causes atherosclerosis which in turn causes AACVD and is responsible for the majority of CVD events [11,23].

The risk of CVD is inversely related to the HDLC concentration. The increase of total HDLC does not necessarily lead to the reduction of CVD risk. The functionality of HDL in the reverse cholesterol transport and in the other athero-protective mechanisms, including the stimulation of endothelic nitric oxide production and antioxidant activation is more important than simply the HDLC concentration in the circulation [23].

Importantly, the elimination of dyslipidaemia results in the single most important benefit against the incidence of CVD in the form of AACVD. The correction of dyslipidaemia is directly related to the decrease in CV risk [1]. Several total CV risk prediction algorithms are conventionally used for total CV risk prediction. The Framingham 10 year total CV risk scoring system is commonly used. The Framingham system is also used in Namibia. It is used to predict the likelihood of a CV event over the next 10 years in a patient. The CV risk scoring systems are commonly based on traditional biomarkers based on lipid profile and

other risk factors. The establishment of CV risk estimation of a lipid profile is part of the total CV risk estimation/prediction. The Framingham risk categories are low, intermediate, high, and very high risk categories [1]. Since the clinical data (which may provide other CV risk factors) is unavailable to the diagnostic laboratory, the patient result interpretation is usually interpreted relative to an intermediate CV risk category. A footnote must be provided to give an interpretation for all the Framingham CV risk categories [10].

2.5 Lipid Based CV risk estimation and treatment assessment

As a result of the high prevalence of CVD in the world and the clear link to dyslipidaemia, usually in the form of elevated LDLC with AACVD, blood is frequently analysed for dyslipidaemia [12,13]. Quantitating the atherogenic lipoprotein particles is the best determinant of CV risk [26]. Atherogenic lipoprotein particles quantities give the best biomarkers for the goals of therapy. However, quantitating the atherogenic lipoprotein particles has logistic and analytic challenges. Routinely, the lipid profile TGs and cholesterol assays (TC, HDLC, LDLC) are used as surrogates or proxies for estimating lipoprotein particle concentrations [26,29].

The routine assessment of cholesterol as a risk factor in CVD development is done using the lipid profile which commonly includes the measured TC, TGs, HDLC, and cLDLC. Lipid profiles report lipid concentrations through measurement of serum concentrations of TC, LDLC, HDLC, and TG. The TC, HDLC, and TG are measured directly. The LDLC, HDLC, and TGs are used as indicators of the concentration of LDL, HDL and VLDL particles respectively. Cholesterol consists of several fractions, the main ones being LDLC, HDLC, IDLC, VLDLC, and chylomicrons. The majority (70%) of the circulatory system cholesterol

is transported by the LDL particles in the form of LDLC fraction [14]. The LDLC concentration has the strongest association with the risk of developing CVD [2,13].

Routinely, laboratories in Namibia and most parts of the world make use of the Friedewald method in calculating the LDLC levels from the measurements of TC, TG, and HDLC. Also, LDLC can be assayed directly using homogeneous methods like the Roche Diagnostic direct homogeneous method [20]. Some few small private laboratories in Namibia use different types of direct homogeneous methods including the Roche method. In addition to LDLC, lipid based calculations like non-HDLC, sdLDLC, and the HDLC/LDLC ration will enrich the diagnosis of dyslipidaemia [15]. The non-HDLC is not used in Namibia and rarely used in the world, the sdLDLC is a new method that is rarely used in the world while some variation of the lipid ratios are commonly used in Namibia and the rest of the world.

2.5.1 LDLC based CV risk estimation and treatment assessment

Of the various cholesterol fractions, LDLC has the highest proportion of cholesterol and transports about 70% of plasma total cholesterol [14]. Research has established that elevated LDLC blood level is the most prevalent type of dyslipidaemia [1]. Although the onset of CVD is consistently related to the presence of a group of CV risk factors, the elimination of hypercholesterolemia (commonly the reduction of elevated LDLC) results in the single most important benefit against the incidence of AACVD and hence CVD [15,21,23].

LDLC is the key factor in the pathogenesis and progression of atherosclerosis and AACVD [26,29]. The serum LDLC concentration is the most powerful clinical predictor among all of the single parameters with respect to atherosclerosis and CVD [35]. There is a strong,

positive correlation between increased level of LDLC and the risk of developing CVD [2,10,13,14,37,38]. Reducing high LDLC levels with LDL particle lowering agents like statins or with therapeutic lifestyle change significantly reduces CV risk [29]. Increased LDLC levels correlate strongly and positively with the extent of the atherosclerotic lesions and the incidence of CVD. The diagnosis and management of adults with hypercholesterolemia and AACVD are largely based on LDLC levels. Therefore, therapies focusing on lipid reduction primarily target the reduction of LDLC [13,35]. LDLC reduction indicates an improvement of the endothelial function, prevention of atherosclerosis, reduction of atherosclerosis progression, and the prevention of plaque rupture [35].

Reports from multiple studies show that the plasma LDLC is lowered by lifestyle changes and lipid lowering drug treatments (LLDTs). Because LDLC is a modifiable risk factor for CVD, its routine measurement is recommended in the evaluation and management of hypercholesterolemia [11]. Therefore, the LDLC levels continue to constitute the primary target of dyslipidaemia screening, AACV risk estimation, initiating and monitoring therapy under the NCEP ATP (III) guidelines and expert panels' recommendations [1,3,21,23].

Consequently expert panels (including the NCEP) and national guidelines use LDLC levels to categorise the patients' risk and to assign corresponding LDLC treatment goals. The main principle of the guidelines is that the higher the patient's risk, the lower the LDLC target needs to be to mitigate that risk [29]. The national guidelines and the expert panels use the LDLC levels as a primary target of cholesterol lowering therapy. As a result, the primary goals of therapy and the cut-points for initiating treatment are stated in terms of LDLC. The recommended LDLC levels validated by the Beta (B)-Quantification (BQ) reference method

are as follows: <2.59 mmol/L (Optimal), 2.59 – 3.34 (Near or Above Optimal), 3.35 – 4.13 (Borderline High), 4.14 – 4.89 mmol/L (High), >4.89 mmol/L (Very High) [37,38]. See Table 2 for LDLC based CV risk categories and the subsequent therapeutic interventions.

The NCEP prescribed stratified serum LDLC ranges used to classify individuals into CV risk categories are very narrow as shown in Table 2 [2,14]. In order to properly classify a patient at risk of developing CVD, LDLC must be measured using a methodology which has an acceptable analytical error (less than 12%). To meet this performance goal, the routine diagnostic laboratories must use methods which are traceable to the CDC reference methods. CDC maintains the Cholesterol Reference Method Laboratory Network (CRMLN) which assists diagnostic analysers and reagents manufactures in validating their methods [23]. Accurate and precise measurements of patients' LDLC are necessary to identify individuals with CV risk and also to determine the necessary intervention in the form of either therapeutic lifestyle change (TLC) or drug therapy (DT) [13]. Table 2 shows the LDLC classification of adults into CV risk categories and the subsequent therapeutic interventions

Table 2: LDLC concentrations used to classify adults at risk of developing CVD and the subsequent therapeutic interventions [19].

	LDLC concentration (mmol/L)	Interpretation	Intervention
1	<2.59	Optimal	None
2	2.59 – 3.34	Near/Above optimal	TLC, DT optional
3	3.35 – 4.13	Borderline High	TLC and DT
4	4.14 – 4.89	High	TLC and DT
5	>4.89	Very High	TLC and DT

DT – Drug Therapy; **TLC** – Therapeutic Lifestyle Change

The therapeutic target for LDLC is dependent on the individual's risk level. The reference ranges used routinely in clinical practice are for patients with a moderate risk from the Framingham risk scoring system.

Most clinical laboratories determine the LDLC using the Friedewald method [14,38]. The LDLC can also be measured directly. The reference method for LDLC separation and measurement is the BQ method which includes analytical ultra-centrifugation, precipitation techniques, and manual procedures which make it tedious, time consuming and difficult to standardise. The BQ method is not feasible for routine laboratory use and it is confined to research and specialised laboratories [38,39].

2.5.1.1 Friedewald method and lipid profile interpretation

The Friedewald method has been the method for calculating LDLC concentrations in many studies and the LDLC cut-off values are based on the data derived from these studies [23]. In this method TC and HDLC are measured using laboratory assays while VLDLC and LDLC are calculated using TG levels.

The following equation was assumed to be correct in a non-fasting blood sample.

TC = HDLC + LDLC + VLDLC + IDLC + Chylomicron + Lp(a)-C + Remnant C [26].

Under non pathological circumstances, when fasting, there are no chylomicrons, remnants (smaller chylomicrons or VLDL particles) or IDL particles. Lp(a)-C levels are negligible. The chylomicrons, remnants and IDL particles are post prandial lipoproteins [26].

Most of the TG is in the chylomicrons, VLDL, and remnants. TC is the cholesterol content of all the lipoproteins. Practically, the LDLC is the cholesterol content of all the IDL and LDL particles. When laboratories report calculated or directly measured LDLC, it is a measure of LDLC and IDLC. Routinely laboratories cannot separate LDLs and IDLs as the separation process is too expensive and most of the time does not confer advantage in the diagnosis. HDLC is the cholesterol content of all of the HDL particles. Remnant cholesterol is the cholesterol content of both of the remnants (chylomicron and VLDL). L(p)a-C is the cholesterol content of LDLC particles that have apo A attached [26].

LDLC is estimated using the Friedewald method as follows:

Calculated LDLC (mmol/L) = TC – [measured HDLC + (measured TG/2.17)], for TG<4.52mmol/L. TG/2.17 is an estimation of VLDLC. Therefore -

cLDLC (mmol/L) = TC - [measured HDLC + estimated VLDLC], for TG<4.52mmol/L [14].

TC = HDLC + LDLC + VLDLC, where VLDLC = (TG/2.17)

The Friedewald method calculated LDLC (cLDLC) has several limitations. The methodological errors accumulate since the method uses three separate lipid fractions of TC, TG, and HDLC. The total analytical error when using the Friedewald method for LDLC is

>12%, which is unacceptably high according to the NCEP recommendations [1,2]. The serum LDLC ranges used to assess the risk of developing CVDs are very narrow (as shown on Table 1), therefore the chances of classifying a patient to an incorrect risk category with a diagnostic method including the Fridewald method are high [2]. Due to narrow reference ranges of the risk categories, the NCEP established that clinical laboratories should use methodologies for measuring LDLC concentrations which have a ≤12% analytical error [2,14,35,40].

A constant cholesterol/TG ratio in VLDL is assumed. VLDL carries most of the circulating TGs and VLDLC is estimated reasonably from measured total TGs (TG/2.17). This is true for TG ≤4.52 mmol/L. This assumption does not hold for TG>4.52 mmol/L. The Friedewald method significantly underestimates the LDLC concentrations when TGs are >4.52 mmol/L and in samples with chylomicrons [41]. However, the accuracy of the Friedewald formula decreases already when TGs are >2.6mmol/L but adequate estimates can still be obtained with TGs up to 4.52mmol/L. An overproduction of a TG-enriched large VLDL causing high generation of sdLDL might lead to overestimation of the VLDLC and underestimation of the calculated LDLC (cLDLC) concentration [23].

In the Friedewald equation it is assumed that most of the TGs are in the VLDL fraction so when post-prandial chylomicrons are abundant, this equation must not be used. The difference between the cLDLC and the direct LDLC (dLDLC) is due to variation in TG, HDLC, and potentially the presence of sdLDL. Routinely when serum TG concentration is >4.52 mmol/L, the LDLC is not calculated using the Friedewald method [1,14,23]. The use of the Friedewald method has been indicated for fasting specimens only. Clinically the

sample of choice for the lipid profile remains a fasting serum sample which refers to 8 – 14 hours usually overnight complete dietary restriction with the exception of water and medication [11,29,41]. The fasting state is essential for TG estimation because the TG remains high for several hours after a fat rich meal, and the Friedewald method used for the calculation of LDLC uses fasting TG value. It has also been reported that, if a non-fasting TG value is used in the method the LDLC; the target of lipid lowering therapy will be significantly underestimated [42]. However calculated LDLC determined using the Friedewald method at TG<4.52 is similar to LDLC measured directly on both fasting and non-fasting lipograms [1].

HDL particles are apo A particles while LDL and VLDL particles are apo B particles. Therefore TC is the sum of cholesterol in the apo B and apo A-1 particles. Therefore: TC = apo B-C + apo A-C.

Conventionally the cholesterol in apo B particles is referred to as non-HDLC. Apo B-C is the cholesterol that is not in the HDL particles. Apo B particles are the atherogenic particles. Non-HDLC is the best surrogate (in the lipid profile) of apo B or atherogenic particles. Therefore: Apo B-C = non-HDLC ≈ LDLC.

In almost all circumstances the majority of the apo B particles are LDL particles. The chylomicrons, VLDLs, IDLs, and remnants have half-lives which range from a few minutes to 6 hours. LDL particles have longer half-lives of 2 – 3 days, thus under most circumstances approximately 90% of the apo B level represents LDL particles. NCEP uses LDLC as the apo B surrogate and non-HDLC as the apo B surrogate when hypertriglyceridemia is present

(when TG > 2.26 mmol/L), non-fasting samples, or in patients with metS [26]. When TG levels are elevated there is delayed catabolism (lipolysis) of TG rich apolipoproteins namely VLDL, chylomicrons, IDLs, and remnants. Reports indicate that non-HDLC is a better surrogate of apo B than is LDLC when serum TG concentration is greater than 0.80mmol/L [26].

Apo B surrogates include TC, LDLC, non-HDLC, TG, and VLDLC. HDLC is the only surrogate of apo A-1. HDL particles contain several (typically 1 – 4) apo A-1 proteins. The relationship between apo A-1 and apo A-1 (HDL) particles is not linear and is not predictable. Therefore the use of HDLC as a surrogate of apo A-1 must be done with caution. All apo B particles contain one apo B molecule per lipoprotein particle. Thus the relationship between apo B and apo B particles is more linear than is apo A-1 to HDLP relationship [26].

For perfect TGs (<1.14 mmol/L), non-HDLC and LDLC are equally good as a surrogate of apo B. However many patients have TGs >1.14mmol/L. In the category 1.14 – 4.52 mmol/L non-HDLC is equal to or better than LDLC as a surrogate of apo B. At TG > 4.52 mmol/L, TC and dLDLC are the only good surrogate of apo B because when TGs are grossly elevated, one may have few but very large VLDL or chylomicrons which grossly elevate the TG level but not the apo B level. Large VLDL particles convey CV risk through rheologic, multiple coagulation, and inflammatory associations. Also the large VLDL particles create atherogenic remnants and sdLDL [26].

Low HDLC is almost always associated with abnormal apo B particles which is characterised by the presence of small LDL, remnant particles, and large VLDL. Therefore low HDLC is an epi-phenomenon alerting one to all of the atherogenic apo B particles (namely small LDL, remnant particles, and large VLDL particles). Treatment in low HDLC states should always be directed first at apo B (LDLC, VLDLC, or non-HDLC) rather than apo A-1 or HDLC. In summary, especially in insulin resistant patients, with unusual exceptions, low HDLC means elevated apo B [26].

2.5.1.1.1 Change in opinion of sample of choice for CV risk estimation

Evidence is indicating that the specimen of choice for lipid profiling is a non-fasting specimen. The American College of Cardiology/American Heart Association (ACC/AHA), European Atherosclerosis Society (EAS), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), Danish Society for Clinical Biochemistry and the United Kingdom (National Institute for Health and Care Excellence) UK NICE guidelines have endorsed a non-fasting sample for routine serum lipid profiling and atherosclerotic associated cardiovascular disease (AACVD) risk estimation [1]. However, a fasting lipid profile is recommended before statin initiation to calculate LDLC and for individuals with non – fasting non – HDLC ≥5.7mmol/L or TG≥4.52 mmol/L as these may be clues to genetic, secondary, or dietary causes of hypertriglyceridemia [42]. A high fat fast food meal on the day of the lipogram will significantly increase the TG and lower LDLC. A single, spurious, non – fasting high (usually very high) TG concentration due to high fat intake preceding blood sampling is followed by other measurements with lower TG concentrations. The use of the Friedewald equation in the presence of chylomicrons underestimates the LDLC [41].

There are perceived limitations on the reliance on non – fasting lipogram [41,42]. Fasting before a lipogram is believed to produce more standardised measurements. In addition the non – fasting lipid profiles are perceived to as providing less accurate measurements that make calculation of LDLC via Friedewald equation invalid [42]. Also, fasting has been a clinical standard. The lipid profiling reference intervals are based on the fasting specimen. It was unclear what values should be flagged as abnormal when using non – fasting rather than fasting serum lipid profile [41].

In the past lipid – lowering trials used fasting lipid measurements. In – order to follow evidence - based practice, fasting blood sampling has often been the standard in CV risk assessment [42]. However, the more recent, in use ACC/AHA, EAS, EFLM, Danish Society for Clinical Biochemistry, the United Kingdom National Institute for Health and Care Excellence (UK NICE), and other bodies recommendations are also based on evidence from numerous large scale population based studies, some, major statin trials and consensus of expert opinions using random, non – fasting blood sampling. These evaluations have provided an evidence base for a change in the conventional practice of using fasting samples [13,21,41].

The non-fasting lipid measurement simplifies blood sampling for patients, laboratories and doctors [41,42]. The non-fasting lipid profiles must be used in the majority of patients including initial CV risk assessment, patients admitted with acute coronary syndrome, if preferred by patient, in children, in diabetic patients, in the elderly, and for patients on stable drug therapy. This improves patient compliance with the lipid testing. The fasting lipid profile must be considered in patients with non-fasting TG>4.52mmol/L, known

hypertriglyceridemia follow-up, recovering from hypertriglyceridemic pancreatitis, starting medications that cause severe hypertriglyceridemia or if additional lab tests are requested that require fasting, for example fasting glucose or therapeutic drug monitoring. The fasting lipid measurement has several disadvantages. Firstly, patients are inconvenienced by having to return on a separate visit for a fasting lipogram. Some patients will default on this essential lipid testing procedure. Also, laboratories are burdened by many patients coming for the fasting tests in the morning. Further, the doctors are burdened by having to review and make decisions on the findings from the fasting lipid profile at a later date. The additional phone calls, emails, follow-up doctor or clinic visit placing extra work on clinical staff is experienced with the fasting not the non – fasting lipid profiling [41].

2.5.1.1.2 Effect of food intake on the serum lipogram and CV risk prediction

Several large – scale population – based studies in USA, Canada, and Denmark, of all age groups, including diabetic patients have now established that serum lipids and lipoproteins changes following habitual food intake are not of statistical and clinical significance in the majority of individuals. The small decrease in TC and LDLC and to a larger extent, the increase in TG are unrelated to habitual/normal food intake in the non – fasting, but to fluid or water intake. A similar drop is actually or may be observed in a fasting lipid profile, where water intake is allowed as the patient desires, before a fasting lipogram. Thus the only way to prevent this drop in LDLC using fasting or non – fasting lipogram testing is to forbid water intake before lipid profile testing. However the fasting lipid profiling procedure in use in Namibia does not prohibit water intake during the fasting procedure. Thus, the water intake has the same effect on fasting and non – fasting lipid profiling [41].

Most people consume several meals in a day. Most people also consume snacks between meals. Human beings exist more in a non-fasting habitual physiological status than the fasting status. However, the NCEP ATP III guidelines recommend that the lipid profile be measured in 8 – 14 hours fasting serum samples. Therefore the conventional fasting lipid profiling measurements may not reflect the daily average plasma lipid and lipoprotein concentrations and the associated CVD risk. Some researchers note that there is no evidence that fasting is superior to non-fasting when evaluating the lipid profile for CVD risk [41].

It has been established that non – fasting lipids are good for the screening of CVD risk. In addition to that, studies that included fasting and or non – fasting individuals gave similar and sometimes superior CVD risk associations for non – fasting compared with fasting lipid profile [41]. The CV risk assessment and the monitoring of lipid lowering drug therapy (LLDT) suggest that non – fasting blood sampling is effective, practical and advantageous in assessing lipid mediated CVD risk and LLDT responses [41].

2.5.1.1.3 Potential for risk of misclassification after transferring from fasting to non-fasting lipid profiling

Transferring from fasting to non-fasting lipid profiles will rarely lead to misclassification of CV risk and error in initiating statin therapy. Statin therapy is based on an individual's total CV risk score including the presence of very high risk, high risk, moderate risk, and low risk factors not just on plasma lipid values in both European and USA guidelines. Therefore minor changes in the lipid profile from fasting to non-fasting conditions will affect only a few individuals regarding the decision to start statin or not. Most guidelines use the LDLC to monitor pharmacological treatment and as goals for treatment. Of note, since the observed reduction in LDLC is due to liberal fluid intake and haemo-dilution rather than to food

consumption, a similar LDLC reduction is likely to occur when using fasting lipid profiles with no restrictions on water intake [41]. Guidelines and consensus statements have concluded from research – based evidence that the cut – off values are the same in the reporting of abnormal non – fasting and fasting TC, LDLC and HDLC. The non – fasting non – HDLC and TGs are insignificantly higher than the fasting cut-off values [41].

Usually in laboratory medicine, results of measured parameters are considered to be abnormal if they exceed the age and gender – specific reference interval. All results below or above these recommended cut – points are flagged with a character to show at a glance that this value deserves attention. Due to widespread unhealthy lifestyle, in most populations, the upper reference cut – point of TC >7.8mmol/L, LDLC >5mmol/L, and a TG>4.52mmol/L are very high and place individuals at increased CV risk. The solution to this is flagging abnormal values based on desirable concentration cut – points rather than reference intervals to identify abnormal test results [41]. However lipids, especially LDLC presents a challenge on the use of desirable concentration cut – points. The LDLC desirable values vary with the individual's total risk between desirable values of <1.8mmol/L in very high risk, <2.59 mmol/L in high risk, <3.0 in moderate risk, and <5.0mmol/L in low risk individuals [19,37] The different values are classified according to the presence or absence of co – morbidities (ACVD, diabetes, chronic kidney disease {CKD}) and other risk factors (age, gender, hypertension, and smoking) [6]. The personalised reporting of desirable values is difficult to implement in laboratory reports because, usually, the clinical conditions and risk factors of the individual patient are not known to the laboratory professional. Therefore a simplified system of flagging abnormal values based on desirable concentration cut – points for moderate risk only is proposed. This flagging must be complemented by more detailed

information on risk stratified cut – points in footnotes on the laboratory reports. Using such flagging will harmonise and standardise the lipid profile results [41].

2.5.1.2 The Roche Diagnostics Direct Homogeneous Method

Despite its limitations, cLDLC is still widely used. LDLC can also be measured directly in all sample categories using homogeneous methods. The current recommendation is that for TG>4.52mmol/L, LDLC must be measured directly. The Friedewald method cannot be used for LDLC estimation in this case [40]. The NCEP, through the Cholesterol Reference Laboratory Method Network (CRLMN) recommends that manufacturers develop direct homogeneous reagent methods which must be evaluated and accepted by the CRLMN laboratories [20]. The Roche Diagnostics direct homogeneous method has passed the CRLMN evaluation, with an acceptable analytical error of <12% [2,35]. An indicator of the reliability of a method is the correct classification of patients by the NCEP medical decision points for LDLC [14].

In addition to passing the CRMLN, the methods must pass validation by routine clinical laboratories. Only then can the methods be adopted. It is unclear how the available direct homogeneous methods compare with the Friedewald and the reference methods. Limited evaluations of the direct homogeneous assays raise questions about their reliability and specificity. Also, some of the studies available were conducted in specialised lipid laboratories and may not be representative of performance in routine clinical laboratories [14,21].

Recently several direct homogeneous methods have been developed for the measurement of LDLC. These methods meet the NCEP criteria for the analytical error [2]. The Roche Diagnostics homogeneous direct LDLC (dLDLC) measurement has advantages. Firstly, the dLDLC method directly measures the LDLC fraction and eliminates the interference of TGs. The dLDLC methods are less sensitive to variation of TG levels compared to the cLDLC method. Secondly, the dLDLC reduces imprecision by employing a single measurement of LDLC instead of the three measurements and two calculations in the cLDLC method of LDLC estimation. Lastly, the dLDLC allows for the measurement of LDLC in all samples for dyslipidaemia including, fasting and non-fasting samples, hypertriglyceridemic samples, and samples from individuals with metS [29,37,38].

The Roche methods appear to better classify individuals into NCEP cut-points than the Friedewald method. However limited evaluations to date raise questions about the reliability and specificity of the direct methods especially in patient samples with atypical lipoproteins like in metS. Available evidence supports recommending homogeneous assays for LDLC to supplement the Friedewald method in those cases where the calculation is known to be unreliable for example when TGs are greater than 4.52 mmol/L, hyperchylomicronaemia, and metS. Before the homogeneous assays can be confidently recommended to replace the Friedewald method in routine practice, more evaluation is needed [14]

Studies comparing classification of patients according to NCEP guidelines using the Friedewald method and the direct homogeneous assays largely indicate that the direct methods are better that the Friedewald method. Unfortunately these studies are usually conducted in experienced speciality lipid laboratories and may not be representative of

performance in the routine clinical laboratories [14]. The evaluation and use of these direct assays in clinical laboratories worldwide has been minimal, and this is largely due to lack of data about their performance and also due to extra costs of the LDLC reagent [2]. The reagent cost advantage favours cLDLC over dLDLC method in the lipid profile. The use of the lipid profile with cLDLC is apparently cheaper than the use of the lipid profile with direct homogeneous methods for example the Roche Direct homogeneous method. With the current reagent cost from the Roche Diagnostics chemistry reagent supplier in Namibia (Biodynamics), the total cost for lipid profile reagents using the Friedewald method (cLDLC) is N$ 2 866.38 per 200 tests. The reagent cost of LDLC and hence the dLDLC is N$ 2 334.50 per 200 tests. Therefore the total cost for the complete lipid profile reagents using the Roche Direct homogeneous method (dLDLC) is N$ 5 200.88 per 200 tests. The cost of the LDLC reagent only is almost the same with the three lipid profile reagents combined (TC, HDLC, and TGs) [43].

The Namibian Association of Medical Aid Fund (NAMAF) 2018 regulated prices per test for the lipid profile tests are N$ 97.20 for TC, N$ 125.60 for HDLC, N$ 144.40 for TGs. Therefore a lipid profile with a cLDLC costs N$ 367.20. All calculated results including the cLDLC are not chargeable. The price per test for the Roche dLDLC is N$100.70. Therefore a lipid profile with a Roche dLDLC costs N$ 467.90 [44].

The data about the performance and the validation of these methods is scarce [14]. The direct homogeneous methods appear to better classify individuals into NCEP cut – points than the Friedewald method. However, the limited evaluations to date raise questions about their reliability and specificity, especially in samples with atypical lipoproteins. Before the direct

homogenous assays can be confidently recommended to replace the calculation in routine practice, more evaluation is needed [13,14,21].

2.5.2 Other Lipid calculations

There is a growing interest in identifying and quantifying other biomarkers, other than the routine lipid biomarkers (such as LDLC) as predictors of CVD. Standard lipid profiling misidentifies CVD risk in a substantial portion of the population [10,18]. The standard lipid fraction measurements, including LDLC should be complimented with other tests. Quantification of more biomarkers such as the non-routine lipid calculations like non-HDLC, sdLDLC, and lipid ratios; and non-lipid analyses like inflammatory cytokines when incorporated in a clinical set up will improve the diagnosis, prognosis and understanding of clinical and premature atherosclerosis pathogenesis [10]. The inclusion of other biomarkers will enhance the accuracy of atherosclerosis risk prediction, enhance the accuracy of outcome prediction, and assist in treatment selection and adjustment in patients with atherosclerosis or CVDs [18].

The in-use traditional cholesterol measurements of TC, LDLC, and HDLC are more accurate in predicting CV risk for individuals at the lower and higher ends of the CV risk spectrum. These in use biomarkers are less accurate in the CV risk estimation in cases at the middle of the risk spectrum and in subclinical cases. The traditional methods of dyslipidaemia diagnosis miss or delay the diagnosis of dyslipidaemia. Undisputed and accurate CV risk estimation biomarkers are lacking. Reduced accuracy of CV risk estimation delays clinical diagnosis, increases CV risk several times, increasing the rates of morbidity (the rate of a disease in a population) and mortality (death). Reduced accuracy of CV risk diagnosis also increases the cost of CVD management. New emerging biomarkers and other markers of atherosclerosis

and the subsequent AACVD must be developed, evaluated, and applied to identify subclinical and asymptomatic patients. Other biomarkers like the lipid ratios, sdLDLC, non-HDLC, and dLDLC must be included in the risk analysis. The new biomarkers must be incorporated into CV risk prediction models to establish whether their addition increases the model's predictive ability. The CV risk prediction models must be updated by incorporating the traditional risk factors, molecular, immunological, genetic, imaging, and other factors for more genuine and reliable CV risk estimation [10,15].

Non-High Density Lipoprotein cholesterol (non-HDLC) is a secondary target for monitoring treatment. SdLDLC is not yet a target for dyslipidaemia diagnosis and treatment monitoring, but NCEP accepts the predominance of sdLDLC as an emerging CV risk factor. The incorporation of sdLDLC into dyslipidaemia diagnosis and treatment targets in the following NCEP ATP edition is expected. [13,19]. Research has established that non-HDLC, sdLDLC, and the HDLC/LDLC ration as individual tests are in some cases superior to LDLC or HDLC alone [15]. Several epidemiologic studies have demonstrated that many patients with CVD or at CV risk have LDLC levels in the same range compared with healthy subjects. Latest evidence reveals that after reaching the therapeutic target for LDLC, a substantial risk for CVD still remains. In this case some calculations including the non – HDLC, sdLDLC, and some lipid ratios may be included as secondary diagnosis and treatment targets for CV risk assessment and lipid lowering drug treatment assessment [15,18,23].

The traditional four measurements of the lipogram can be supplemented with lipid calculations like non – HDLC, sdLDLC, and various lipid ratios (like HDLC/LDLC) without additional costs since these are calculations based on the four lipid measurements. In addition

to that, the lipid calculations enrich the lipid characterisation of each patient with the possibility of a better discrimination of CV risk, particularly among groups at intermediate CV risk [15].

2.5.2.1 Non-HDLC

Elevated LDLC remains the main target of CV risk estimation and the main target of LLDT in the prevention of CVD [17,45]. The individual strong links between CV risk and LDLC, Lp(a) cholesterol, and IDLC are well established. LDLC, Lp(a) cholesterol, and IDLC are included in the routine cLDLC [16]. Residual CV risk has been noted following the correction or lowering of LDLC following LLDT. The residual risk phenomenon calls for another metric in dyslipidaemia management in addition to LDLC. Evidence show that non-HDLC has better discriminative ability beyond LDLC, enabling non-HDLC to better estimate CV risk and provide an accurate therapeutic target [16,17,46]. It has been proven that the risk of CVD remain high in patients who have attained the NCEP and other guidelines-recommended LDLC goals. In a 2012 publication, at least thirty three percent of the United States of America dyslipidaemia patients were showing persistent CV events despite the lowering or correction of the elevated LDLC [46]. Examining non-HDLC picks the residual CV risk not picked by LDLC in treatment monitoring in some patients. Also it has been observed that the incidence of metS decrease the accuracy of CV risk prediction when LDLC is used as the lipid CV diagnostic tool alone, whereas non-HDLC, apo B, LDLP retain the predictive capabilities in this patient population [17,37,46]. The non-HDLC, apo B, and the LDLP account for an atherogenic spectrum more relevant to the increasingly diabetic and obese global population.

Some studies like the Incremental Decrease in End Points through Aggressive Lipid Lowering (IDEAL) trial showed that elevated non-HDLC and apo B levels were better predictors of adverse CV outcomes on patients who were on dyslipidaemia treatment than LDLC [17]. Furthermore, non-HDLC is closely linked to CV risk. Non-HDLC is more sensitive in predicting atherosclerosis progression, CV events, CV risk outcome, and treatment outcome [16,17]. Some researchers established that non-HDLC is as good as or better than LDLC levels in predicting CV risk [47]. Other researchers established that non-HDLC is a better CV risk marker than LDLC [17].

Non – HDLC is a secondary CVD risk predictor (from several guidelines including NCEP and consensus papers). The current NCEP ATP III guidelines recommend that non-HDLC be used as a secondary target of LLDT in individuals with hypertriglyceridemia (TGs >2.26 mmol/L) [16,17]. Some researchers have found that non-HDLC levels correlate closely with LDLC levels in persons with normotriglyceridemia (TG <2.26 mmol/L) than those with triglyceride elevations (>2.26 mmol/L). Patients with hypertriglyceridemia have the greatest discrepancy between LDLC and non-HDLC levels. Unlike the cLDLC, the non-HDLC calculation is not affected by high levels of triglycerides. Patients with elevated TG are expected to benefit more from the non-HDLC targeted strategy. For hypertriglyceridemia, hyperchylomicronaemia, non-fasting or samples from cases with atherogenic lipid triad, non-HDLC may be determined in place of LDLC [16,17]. In the 2008 consensus statement by the ACC Foundation and the ADA, no TG cut-off level was defined for calculating non-HDLC. Some researchers found that non-HDLC levels had the strongest relationship with sdLDLC levels compared with other measurements. Some recent studies suggest that the predictive value of non-HDLC for CV risk and CV mortality is better than that of LDLC [17,18].

LDLC does not account for the entire cohort of atherogenic lipid particles within the systemic circulation. Non-HDLC is a more comprehensive measure of atherogenic lipids than the LDLC level. Non-HDLC is the sum of all atherogenic cholesterol fractions in serum. Non-HDLC encompasses cholesterol from Lp(a) cholesterol, LDLC, and IDLC that are traditionally included in the LDLC values calculated by the Friedewald method, and also cholesterol contained in the atherogenic TGs-rich lipoproteins such as VLDL and VLDL remnants [10,16]. In a non-fasting sample, non-HDLC includes Lp(a) cholesterol, LDLC, IDLC, VLDLC and cholesterol contained in other atherogenic TG-rich lipoprotein remnant particles. In a fasting sample, non-HDLC is defined by the concentration of LDLC and VLDLC. Non-HDLC represents the atherogenic cholesterol, the cholesterol that can build up in the arteries, form the atherosclerotic plaque, and cause narrowing of the blood vessels and blockages. Therefore, non-HDLC level is a more comprehensive measure of lipoprotein atherogenicity than LDLC. Non-HDLC is therefore an independent CV risk factor and a good biomarker for CV risk estimation and treatment guide [16]. Therefore, the treatment of non-HDLC is grounded in a more holistic principle of dyslipidaemia management than LDLC treatment [16,17]. All the lipoprotein particles included in the non-HDLC calculation carry a single molecule of apo B on their surface. Non-HDLC is a better correlate of apo B than LDLC in both normotriglyceridemia and hypertriglyceridemia [16].

Non-HDLC is easy to calculate. Non-HDLC is calculated by subtracting HDLC from TC. Non-HDLC = TC – HDLC. Non-HDLC incurs no additional testing cost to the health care system. No additional new tests need to be ordered. [16,17,46]. Also the non-HDLC usage remains within the familiar lipid-centric framework with targets relevant to LDLC goals [46].

In contrast to the cLDLC levels which are traditionally and routinely calculated by the Friedewald method and requires a fasting blood sample, according to some guidelines like the NCEP, non-HDLC is calculated from either fasting or non-fasting blood samples without the need for TGs measurement. Also, non-HDLC does not make any assumptions about lipoprotein composition [10,15.16,17]. The national guidelines including the NCEP and the expert panels use the non-HDLC as a secondary target for CV risk estimation and cholesterol lowering therapy assessment. The recommended non-HDLC CV risk based categories and the subsequent therapeutic interventions are shown in Table 3

Table 3: Non-HDLC concentrations used to classify adults at risk of developing CVD and the subsequent interventions [17,19].

	Non-HDLC levels (moll/L)	**Result interpretation**	**Intervention**
1	<3.37	Optimal	None
2	3.37 – 4.12	Near/Above Optimal	TLC, DT optional
3	4.13 - 4.90	Borderline High	TLC and DT
4	4.91 – 5.7	High	TLC and DT
5	>5.7	Very High	TLC and DT

DT – Drug Therapy; **TLC** – Therapeutic Lifestyle Change

The non-HDLC LLDT target is 0.78 mmol/L above the LDLC treatment target. Like the LDLC target values, the non-HDLC therapeutic target values are also dependent on the individual's total risk level. Non-HDLC represents improved CV risk assessment over LDLC [16,17].

Elevated levels of non-HDLC in combination with normal levels of LDLC identify a subset of patients with elevated levels of LDL particle number, elevated apo B concentrations, and sdLDLC. Measuring apo B and LDL particle concentration would add expense, it is not standardised, and is not currently endorsed by NCEP and other major national cholesterol treatment guidelines. Also the particle-centric framework of apo B and LDLP is less understood by most health care providers [17,46]. The use of non-HDLC avoids the use of expensive tests that measure LDL particle number, apo B concentration, or LDL phenotype/morphology. The use of non-HDLC improves patient care without increasing the cost of healthcare. The non-HDLC calculation in lipid profiling is endorsed by the current NCEP and other guidelines [17].

The LLDT (statins, fibrates and others) lower the LDLC, TGs, and increase the HDLC levels to varying degrees depending on the underlying lipid abnormality [16]. The LLDT also lowers the non-HDLC in higher doses or in LLD combination therapy. The LLDT non-HDLC reductions subsequently correlate with the CV morbidity and mortality [16]. Although non-HDLC is a better maker of CV risk than LDLC, the LLDT goal attainment for non-HDC is inferior to that of LDLC [17].

Possible explanations for the poor non-HDLC, LLDT goal attainment [17]

i) Deficiencies in the health care providers' knowledge of the importance of non-HDLC
ii) Deficiencies in the health care providers' knowledge of how to calculate the non-HDLC.
iii) Deficiencies in the health care providers' knowledge of the treatment goals for the non-HDLC.

iv) Problems with the patients' tolerance of or compliance with higher doses of LLD or with the addition of LLDs from other classes (which is often needed to attain non-HDLC goals).

v) The absence of the non-HDLC on the routine lipid profile report.

More clinical trials that include a broad spectrum of patients with dyslipidaemia, inclusion of non-HDLC in the lipid profile, audit and feedback to healthcare providers about the non-HDLC treatment goal attainment performance are needed to directly compare an LDLC lowering strategy with a non-HDLC lowering strategy to determine whether the non-HDLC could lead to greater reductions in CVD events [16,17].

The increase in the incidence of metabolic syndrome decreases the accuracy of risk prediction for CVD when LDLC is used for that purpose. Non-HDLC retains a good predictive capability in the metabolic syndrome patient population. Elevated levels of non-HDLC are treatable by use of currently available lipid lowering agents, as well as lifestyle modification [17]. The ACC advices health care providers to use LDLC together with non-HDLC to maximise cost-effective approach to CV risk assessment and treatment [46].

2.5.2.2 4

The traditional lipid profile measurements are more accurate at predicting CV risk for cases at the lower and higher ends of the CV risk spectrum. Recent data is proving that HDLC/LDLC ratio is an accurate predictor of CV risk. The Helsinki, PROSPER, PROCAM, and the Framingham studies and other recent data have established that the HDLC/LDLC ratio is a better CV risk predictor than the LDLC or the HDLC alone [15,47]. Several studies have also demonstrated that the HDLC/LDLC ratio is an excellent way to monitor the impact

of lipid lowering therapies [15]. Following therapeutic intervention, changes in the HDLC/LDLC ratio have been shown to be a better indicator of CVD risk reduction than changes in the absolute levels of lipids (for example LDLC) and lipoproteins [15].

The dissemination of the lipid ratios is limited because the critics of introduction of the ratios argue that it is not yet clear whether the addition of the emerging lipid ratio parameters to the conventional lipid measurements will translate into clear clinical benefit, or will confuse the medical practitioners over the clinical decision frame. Contrary to this, studies have affirmed the lipid ratios' capability to add CV risk discriminative capacity to the individual lipid parameters. Also, the lipid ratios are more favourable than other lipid analyses like apolipoproteins which are more expensive and more difficult to operationalize [15]. The use of lipid ratios in clinical practice is strongly recommended, both in risk stratification and in monitoring the effectiveness of LLDT. In this study the HDLC/LDLC ratio (among other lipid ratios) was used. The NCEP (2001) recommended HDLC/LDLC ratio cut-off is 0.4 [15]. LLDT must be initiated for HDLC/LDLC <0.4. The ratio is calculated from two parameters which are directly measured. This lowers the analytical error, unlike the cLDLC. Also the two parameters used in the ratio represent the actual bidirectional cholesterol traffic, in and out of the arterial intima in a way that the individual LDLC and HDLC levels cannot account for [15]. The HDLC/LDLC ratio cut-off value = 0.4. LLDT is initiated for HDLC/LDLC <0.4

2.5.2.3 sdLDLC

The changes in lifestyle have increased the prevalence in metS. The metS elements/conditions include obesity and type 2 diabetes [46]. These conditions demonstrate different lipoprotein distributions from traditional dyslipidaemia. The LDL particle size has

shifted towards smaller as compared to the larger LDLP size. Also the LDL particle size in patients under treatment has shifted towards smaller as compared to the larger LDLP size [18]. LDLP is the number of LDL particles in a unit volume of blood. This concentration represents all sized LDL particles namely large, intermediate, and small [23,26]. Routinely, sdLDLP is the sum of small and intermediate LDLP per unit volume of blood. The predominance of sdLDLC in the LDLC complex correlates directly with the serum levels of TG, inversely with serum HDLC levels, normal or even low LDLC concentrations. This combined lipid abnormality constitutes the atherogenic lipoprotein phenotype also known as the atherogenic triad which has an important role in the development of atherosclerosis. SdLDL is an independent CVD risk factor, independent from LDLC because patients with CVD may have LDLC within the reference interval, but increased levels of sdLDL. The use of LDLC alone as a diagnostic tool in dyslipidaemia would lead to CV risk misclassification in cases with metS conditions and those under treatment [18,23,26,46].

Research findings are supporting the inclusion of sdLDLC as a secondary target for the diagnosis and treatment monitoring in CV risk assessment. The LDLC calculations only are not sufficient anymore for accurate CVD risk assessment. Numerous epidemiologic and pathologic studies have indicated that increased sdLDL levels are closely associated with increased CV risk. NCEP accepts the predominance of sdLDL as an emerging CV risk factor. [18,23].

The cholesterol concentration carried on sdLDL was found to be significantly higher in severe CVD than in the mild disease. The sdLDLC concentrations are associated with the severity of CVD independent of the levels of LDLC, HDLC, apo B, and non-HDLC. Also,

sdLDLC may be a good biomarker to assess response to therapeutic interventions in patients [18].

Although sdLDL has been recognised as a powerful predictor of atherogenesis, there has been no standard assay procedure in general clinical use. Most sdLDLC methodologies are expensive, time consuming and technically demanding, making them too laborious for routine clinical practice. An equation was developed from the common measure of non-HDLC, dLDLC, and cLDLC to estimate sdLDLC [19].

sdLDLC = 0.575(non-HDLC) + 0.417(dLDLC) – 0.724(cLDLC) – 0.306, all measurements in mmol/L.

The reference ranges used in this research are adopted from Framingham study [18,48,49]. These reference ranges are used to classify individuals in CV risk categories as shown on Table 4.

Table 4: sdLDLC concentrations used to classify adults at risk of developing CVD and the subsequent interventions

	sdLDLC concentration (mmol/L)	Interpretation	Intervention
1	<0.5	Optimal	None
2	0.5 – 1.0	Borderline High	TLC and DT
3	>1.0	High	TLC and DT

DT – Drug Therapy; **TLC** – Therapeutic Lifestyle Change

The calculated sdLDLC provides a cost effective method for screening patients for the risk of CVD. There is a strong linear relationship between measured and calculated sdLDLC values.

This inexpensive calculation of sdLDL particles preselects patients who would benefit from a more definitive sub fraction work-up. [18].

Some research findings showed that non-HDLC levels have the strongest relationship with sdLDLC levels compared with other lipid measures. Observational and interventional studies indicate that non-HDLC is a better lipid biomarker than LDLC in the diagnosis of CV risk and assessment [18,46]. The strong association of non-HDLC and the sdLDLC and the superiority of the non-HDLC over the LDLC make the non-HDLC a better lipid CV risk biomarker than LDLC [18].

2.6 Non-lipid based techniques for CV risk estimation

The new technologies allow the quantification of dyslipidaemia associated CV risk in other ways besides the routine cholesterol quantities. The other dyslipidaemia associated CV risk estimation technologies include the nuclear magnetic resonance (NMR), the gradient gel electrophoresis, and the vertical auto-profile (VAP) test. The newer tests provide information beyond the standard cholesterol values [29].

The lipoprotein particles that carry cholesterol and TGs are the direct mediators of atherosclerosis and AACVD. It is more specific and sensitive to measure lipoprotein particles than measuring cholesterols and TGs. Due to analytical challenges in measuring lipoprotein particles, cholesterol and TGs assays have been used as surrogate tests used as a basis to estimate lipoprotein particle concentrations. Serum TGs, LDLC, and HDLC values serve as surrogate measurements for VLDLP, LDLP, and HDLP respectively [29].

2.6.1 Nuclear Magnetic Resonance (NMR)

The nuclear magnetic resonance (NMR) method separates lipoprotein particles according to their size into total number of particles (LDLP and HDLP), LDLP and HDLP subpopulations. The NMR Lipoprofile test measures the number of particles present in a variety of lipoprotein classes and subclasses including total LDLP number, small LDLP number, small HDLP number and large HDLP number. The mean lipoprotein particle sizes of LDLP, HDLP, and VLDLP are also provided. The lipoprotein particle subpopulations are associated with the lipoproteins functionality, therefore the measurement of lipoprotein particle sizes is a better approach than the approach of cholesterols and TGs measurements. Multiple outcome trials show strong and independent relationship of LDLP with CV events [29].

The superiority of LDLP as a measure of CV risk estimation is seen when LDLC and LDLP results are discordant. In this case, CV risk tracks closely with LDLP but not LDLC. For this reason many expert panels including the ACC, American Diabetes Association (ADA), and the American Association of Clinical Endocrinologists (AACE) have recommended the use of LDLP targets in addition to cholesterol (LDLC and non-HDLC) targets to optimise individual management of moderate and high risk individuals. However, the NMR demands the use of expensive, special equipment and expertise. These methods are not suitable for a routine African diagnostic laboratory [23].

2.6.2 Vertical Autoprofile (VAP) Test

The VAP test categorises LDLC and HDLC by relative size. In this case several LDLC and HDLC subclasses are reported based on cholesterol content. VAP also measures cholesterol fractions that current lipid profile ignores e.g. VLDLC, IDLC, Lp(a) C, and apoB 100 [29].

Routine diagnostic laboratories which cannot afford the expensive and demanding methods like the NMR, gel electrophoresis, and the VAP may use verified equations for particle calculation for example the method put forward by Srisawasdi et al [18].

Whenever cholesterol (LDLC) and LDLP measurements are discordant, CV risk tracks well with LDLP number. As a result several expert panels including the ACC and ADA advice treating to LDLP targets(either NMR, LDLP or measured apo B) in addition to LDLC and non-HDLC to optimise management of moderate and high risk individuals [29].

2.6.3 Inflammatory biomarkers for predicting CVD

The CV risk prediction must include the recently technologically generated diagnostic biomarkers such as inflammatory biomarkers. The inflammatory biomarkers, in isolation or in combination with other biomarkers, predict CV risk, CVD elements, and status of morbidity [10,15]. There is need to validate and standardise the emerging biomarkers in CV risk prediction and monitoring of therapeutic response [10]. The treatment of dyslipidaemia is moving beyond the reduction of LDLC alone to include the control of inflammatory components [30]. Conventional lipid risk prediction algorithms are available and, work well in the presence of major CV risk factors identified in diseased population. Standard lipid profiling misidentifies some CVD risk statuses in a substantial portion of the population [10,22]. However authentic, fast, reliable, and accurate biomarkers of prediction and confirmation of CVD at the subclinical level, in time to start management are lacking. Also the missing of residual risk after treatment with LLD, a problem currently exposes patients to risk. Delayed CVD risk prediction delays clinical diagnosis, increases CV risk, and results in increased mortality and morbidity. Also, a false treatment success increases CV risk and result in increased mortality and morbidity [1].

The pathology of CVD is complex. Many biological pathways including inflammation are involved in the pathogenesis and progression of CVD. Atherosclerosis is also controlled by inflammatory and chemotactic biomarkers and hence these biomarkers are considered potential biomarkers for predicting patients at risk of CVD development, recurrent CV events in CAD patients, monitor efficacy of treatment for patients at risk of CVD developments and those with CVD [10,33,34].

Due to complexities of CVD pathogenesis there is no single biomarker available to estimate absolute risk of future CV events. Furthermore not all biomarkers are equal, the functions of many biomarkers overlap, some biomarkers offer better prognostic information than others, and some biomarkers are better suited to identify or predict the pathogenesis of particular CV events. The identification of the most appropriate set of biomarkers can provide a detailed picture of the specific nature of the CV event [34].

Atheromatous plaque development involves dysfunction of activated endothelial cells which produce adhesion molecules that interact with inflammatory cells, abundant presence of inflammatory cells (monocyte derived macrophages and T lymphocytes) at the site of rupture or superficial erosion, and monocyte derived macrophages secretion of cytokines, chemokines, growth factors, and disintergrins. These biomarkers lead to activation and proliferation of smooth muscle cells, lesion progression, and finally to the weakening of a vulnerable plaque by matrix degradation of its fibrous cap [34].

Once inflammation has been triggered and cytokine release is initiated at the onset of atherosclerotic lesion development, a number of factors that are found in the atherosclerotic plaque participate in maintaining and amplifying cytokine production, including adipokines, reactive oxygen species, and pro-inflammatory cytokines. The reactive oxygen species oxidise cholesterol [10,33]. The inflammatory response causes pathological changes in cells long before marked and advanced symptoms become apparent and diagnosis is made [10,33].

2.6.3.1 Cytokines

Cytokines are low molecular weight proteins produced de novo by immune cells and non-immune cells in response to an external stimulus. Cytokines regulate host responses to infection and inflammation. Cytokines are also involved in regulatory immune response. Cytokines generally act over short time spans and at very low concentrations by binding to specific high affinity membrane cytokine receptors which signal the cell to alter their behaviour (gene expression). Cytokines induce biological effects in selected microenvironments in the body even at minute quantities. Responses to cytokines include increase or decrease in expression of membrane proteins (including cytokine receptors), proliferation and secretion of effector molecules. The action of cytokines is pleiotropic and redundant. Cytokines are powerful mediators in altering the immune response including inflammation. Assessment of cytokines in body fluids provides information in understanding of disease process and designing treatment strategies. The biology of cytokines is very complex due to a variety of factors such as a short half-life, low plasma concentrations, pleiotropy, redundancy, and the fact that much more still remains to be learned about cytokines [50].

The pathogenesis of atherosclerosis involves a complex interplay between cytokines, chemokines, and adhesion molecules, leading to monocyte infiltration and multiple other leukocyte responses within the arterial wall. Studies are showing an association between Interleukin-1 (IL-1), IL-6 and Tumour Necrosis Factor-Alpha (TNF-α) with elevated serum LDLC levels and the pathogenesis and progression of CVD [40]. Currently there is little evidence to support the introduction of these non-lipid biomarkers as diagnostic tools in risk stratification and management of CVD. The use of these non-lipid biomarkers in diagnosis will contribute to the management of individuals with CV risk but without manifest CVD and those with established CVD [16].

The inflammatory biomarkers, in isolation or in combination with other biomarkers, predict CV risk, CV types, and status of morbidity [10]. There is need to validate and standardise the emerging biomarkers in CV risk prediction and monitoring of therapeutic response [10]. The treatment of dyslipidaemia is moving beyond the reduction of LDLC and the alteration of some lipid components alone to include the control of inflammatory components [30].

3. RESEARCH METHODS

3.1 Research Design

A convenient sampling, quantitative and comparative study of the calculation (Friedewald) and direct measurement (Roche Diagnostics direct homogeneous) methods for LDL, the non-HDLC, the HDLC/LDLC ratio, and the sdLDLC was carried out at CLS Pathology Xpress Laboratory. A total of 123 serum samples were collected from CLS Pathology Xpress Laboratory, High Care Laboratory, NamPath Laboratory, and Glowshot Laboratory in Windhoek, Namibia.

3.2 Study Population

The target population comprised of private patients suspected of hypercholesterolemia or other lipid abnormalities who were visiting private medical practitioners of whom a fasting or a non – fasting lipid profile was ordered.

3.3 Sample

In this study, a total of 123 secondary fasting and non-fasting serum samples from adult patients (above 18 years of age) received at the participating laboratories in Windhoek, where a full lipid profile was requested were considered for the study. The serum samples were collected and assayed over the period September 2017 until May 2018. Only non-haemolytic, non-icteric, and sera with TG ≤4.52 mmol/L were considered for this study. TG concentrations >4.52 mmol/L are not applicable to the Friedewald method. Convenience sampling was used in selecting samples which met the criteria. Two serum samples were excluded from the study because the TG levels were greater than 4.52mmol/L. Therefore 121 serum samples were used in the study.

3.3.1 Sample size

A convenient sample size was determined using The Survey System sample size calculator [51]. The sample size was determined using the 95% confidence level, confidence interval of 10, and a target population of 2000. The sample size was calculated at 92. This sample population is in the same range with related studies [13,37,39]. The number of samples used in this study (121) exceeded the number of the calculated target sample size of 92

3.4 PROCEDURES

3.4.1 Ethical approval and research ethics

Ethical approval for the research was granted by the Ministry of Health and Social Services (MoHSS) and UNAM Research Council (UREC) for the use of patients sera (Appendices 9.1, 9.2, and 9.3). Blood samples were collected by qualified and registered medical personnel. The co-supervisor and the researcher are also registered with the Interim Health Professions Council of Namibia (HPCNA) medical regulatory board (Appendices 9.4 and 9.5 respectively). All the participating laboratories are certified by the MoHSS to handle human samples and information. Secondary samples were used for the research so informed consent was not sought as patient names were not used and patient identification was not disclosed. Blood samples will not be used for future research, and no commercial profit will be gained. The study did not expose participants to risk and caused no harm to individuals [52,53,54,55]. All patient information and the subsequently generated results were treated with confidentiality. The research patient serum samples identification was based on laboratory generated numbers, not names. After analysis the samples were disposed of using standard procedures for disposal of medical waste [57]. The final report of the study was shared with the MoHSS, UREC and the research supervisors.

3.4.2 Sample preparation [56]

Samples were collected from CLS Pathology Laboratory, and other participating laboratories in Windhoek. Sufficient volume (3 – 5ml) of fasting or non-fasting venous blood was drawn into labelled Serum Separator Tubes (SST) and the blood was allowed to clot at room temperature for 30 to 60 min. In cases where the samples for CLS were collected outside CLS Pathology Laboratory, the samples were transported in tightly closed cool 5L specimen boxes with one or two ice packs (routine cold chain procedure) to the laboratory. A research worksheet was given to participating laboratories for data capture (Appendix 9.7). Serum was separated from the red blood cells within 2 hrs of collection. After clotting, the specimens were centrifuged at 3 000g for 10 min. In cases where the sera were prepared at other laboratories, the sera were transported in tightly closed cool 5L specimen boxes with one or two ice packs (routine cold chain procedure) to CLS Pathology Laboratory. The serum was transferred into 2 labelled cryovials immediately after centrifugation or after lipid profile analysis. The serum sample aliquots were securely contained in cryovials that prevent breakage, leakage, and evaporation. Sera were assayed for lipid profile at CLS Pathology Laboratory immediately after separation, on the same day of specimen collection (after room temperature or 4 – 8 °C storage), or within 7 days at 4 – 8 °C storage.

3.4.3 Quality Control (QC)

3.4.3.1 CLS Cobas c111 Instrument Validation

The Cobas c111 chemistry analyser at CLS laboratory was validated in method based and instrument based performance against other analysers under the Thistle/Randox EQA program. The CLS Pathology Xpress Laboratory c111 passed validation for the lipid profile tests (Appendix 9.8.1).

3.4.3.2 Inter-laboratory (QC)/External Quality Assurance

The performance of the CLS Laboratory Cobas c111 was compared with that of Medical Diagnostic Laboratory and High Care Laboratory. The two laboratories are enrolled on EQA and are performing well. The CLS Pathology Laboratory inter-laboratory EQA performance was comparable with those of the other two EQA enrolled laboratories (Appendix 9.8.2).

3.4.3.3 Internal (QC)

The procedure prescribed two internal QC material levels were run with every analytical run. The IQC results were acceptable (Appendices 9.9.1 and 9.9.2).

3.4.4 Lipid Biomarker CV risk estimation

3.4.4.1 Friedewald Method (LDLC calculation)

For each serum sample; TC, TG, and HDLC were directly measured using a Cobas c111 auto – analyser from Roche Diagnostics (Mannheim, Germany). The Cholesterol Gen.2 enzymatic colorimetric method, Triglyceride enzymatic colorimetric method, and the HDL-Cholesterol plus 3rd generation direct homogeneous enzymatic colorimetric method were used in the assay of TC, TG, and HDLC respectively. The reagents, calibrators, and controls were used according to the manufacturer's instructions [11]. The serum concentration of LDLC was calculated using the Friedewald method:

LDLC (mmol/L) = TC – (HDLC + VLDLC) and VLDLC (mmol/L) = (TG/2.17) for TG values less than 4.52 mmol/L [37].

The serum sample; TC, TG, HDLC, and cLDLC values were recorded on a lipid biomarker results worksheet (Appendix 9.12).

3.4.4.2 The Roche Diagnostics direct homogeneous method (LDLC measurement)

For each serum sample, the LDLC were directly measured using a Cobas c111 auto – analyser from Roche Diagnostics (Mannheim, Germany). The serum sample dLDLC values were recorded on a lipid biomarker results worksheet (Appendix 9.12). The LDL-Cholesterol Gen.2 homogeneous enzymatic colorimetric method was used in the assay of LDLC [35].. The reagents, calibrators, and controls were used according to the manufacturer's instructions. The LDLC plus 2^{nd} generation method is based on the selective micellary solubilisation of LDLC by a non-ionic detergent and the interaction of a sugar compound and other lipoproteins other than LDL (VLDL and chylomicrons). In the presence of Mg^{2+}, the sugar compound markedly reduces the enzymatic reaction of the cholesterol in the VLDL and chylomicrons. The combination of a detergent with a compound sugar enables the selective determination of LDLC in serum [35].

3.4.4.3 Non-HDLC calculation

The serum concentrations of TC and HDLC were measured as described and were used for calculating the non-HDLC as follows:

Non-HDLC = TC – HDLC [16,17].

The serum sample non-HDLC values were recorded on a lipid biomarker results worksheet (Appendix 11.12). The non-HDLC was normalised by adjusting non-HDLC to non-HDLCA by subtracting 0.78 mmol/L from non-HDLC. The adjustment allows for direct numerical comparisons between the LDLC and the non-HDLC values [45].

Non-HDLCA mmol/L = Non-HDLC – 0.78 [45].

3.4.4.4 HDLC/LDLC ratio calculations

The direct measurement of concentrations of TC, HDLC, LDLC and TGs were performed as described above. The estimation of LDLC fraction was calculated using the Friedewald method as described above [35,37].

In this study, the HDLC/LDLC ratio was calculated on serum samples with TGs less than 4.52mmol/L and a normal/clear serum appearance. However, the HDLC/dLDLC may be calculated in all TGs concentrations while the HDLC/cLDLC may only be done in TGs concentrations less than 4.52 mmol/L because the cLDLC is only valid in TG levels <4.52 mmol/L [41]. The serum sample HDLC/LDLC ratios were recorded on a lipid biomarker results worksheet (Appendix 9.12)

3.4.4.5 SdLDLC calculation

The differences in the Friedewald calculated LDLC (cLDLC) and the Roche Diagnostics direct homogeneous enzymatic colorimetric method LDLC (dLDLC) in the same serum sample is a result of the presence of sdLDLC, TG and LDLC. This observation has led to the development of various equations in an attempt to calculate sdLDLC, an independent and emerging CV risk factor. In this study, the difference between the cLDLC and the dLDLC was used to calculate sdLDLC, employing the equation put forward by Srisawasdi et al (2011) [18];

SdLDLC (mmol/L) = 0.575(non-HDLC) + 0.417(dLDLC) – 0.724(cLDLC) – 0.306. The serum sample sLDLC values were recorded on a lipid biomarker results worksheet (Appendix 9.12)

3.5 DATA ANALYSIS

The statistical package of social sciences (SPSS) version 24 and Microsoft Excel were used to analyse the descriptive statistics. The lipid methods correlation coefficients, CV risk categories, and paired t test analyses were used to establish the correlation, the clinical discordance, and the significance of the differences between the cLDLC, dLDLC, and the non-HDLC/non-HDLCA concentrations. The in-use NCEP ATP III guidelines established that non-HDLC CV risk categories and treatment targets are 0.78mmol/L above the LDLC values for CV risk classification. The relationship between cLDLC, dLDLC, and non-HDLC/non-HDLCA were determined statistically using the correlation coefficient and the paired t test analyses. The relationship was also determined clinically based on cLDLC, dLDLC, and the non-HDLC/non-HDLCA numerical values and the ATP III CV risk categories [45]. Assays specific methods were used to quantitate non-LDLC and non-HDLC biomarker levels in sera and classify the patient sera into CV risk categories based on those biomarkers. The non-LDLC non-HDLC/ non-HDLCA biomarkers were compared with LDLC and non-HDLC values in their classification of adults into CV risk categories.

4. RESULTS

A total of 123 serum samples were collected and a full lipid profile (TC, TGs, HDLC, and LDLC) was done on all the serum samples. Forty six (46) serum samples were collected from CLS Pathology Xpress Laboratory, 7 serum samples were collected from High Care Laboratory, 59 serum samples were collected from Nam Path Laboratory, and 11 serum samples were collected from Glowshot Laboratory. Two samples were excluded from the study due to hypertriglyceridemia TG>4.52 mmol/L The 2 samples excluded from the study came from Nam Path Laboratory. A total of 121 samples were used in the study. The study sample population comprised of 54 adult males (45%) and 67 adult females (55%).

4.1 A comparison of cLDLC, dLDLC, non-HDLCA, and non-HDLC using linear regression analysis.

The linear regression analysis was used in the analysis of the LDLC and the non-HDLC/non-HDLCA serum results to establish whether the 3 methods of cLDLC, dLDLc, and the non-HDLC/non-HDLCA gave LDLC/non-HDLC values which were significantly different. The linear regression graphs and the subsequent values are shown on Figures 3, 4, 5 6, 7, and 8 below. On all the correlations; cLDLC and dLDLC (Figure 3), non-HDLCA and non-HDLC (Figure 4), cLDLC and non-HDLCA (Figure 5), cLDLC and the non-HDLC (Figure 6), non-HDLCA and dLDLC (Figure 7), and non-HDLC and the dLDLC (Figure 8) the results correlations were high and linear suggesting the results were not significantly different.

Figure 3 shows the correlation between cLDLC and dLDLC concentrations obtained from the study serum samples. The regression statistics obtained between the cLDLC (y) and the dLDLC (x) values were y = 1.0761x + 0.0416, with a correlation coefficient of 0.94 shows that the cLDLC and the dLDLC highly correlate in a linear manner.

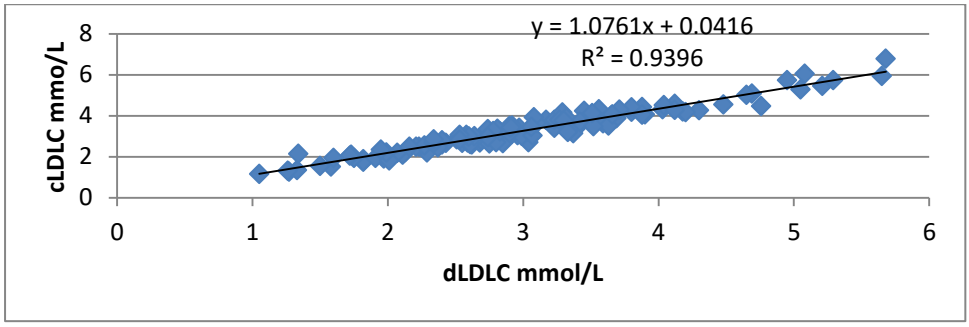

Figure 3: A linear regression analysis of the cLDLC and the dLDLC methods

Figure 4 shows the correlation between non-HDLCA and non-HDLC values. The regression statistics obtained between the non-HDLCA (y) and the (non-HDLC) (x) values were y = x – 0.78, with a correlation coefficient of 1 shows that the non-HDLCA and the non-HDLC highly correlate in a linear manner. These methods can be used interchangeably.

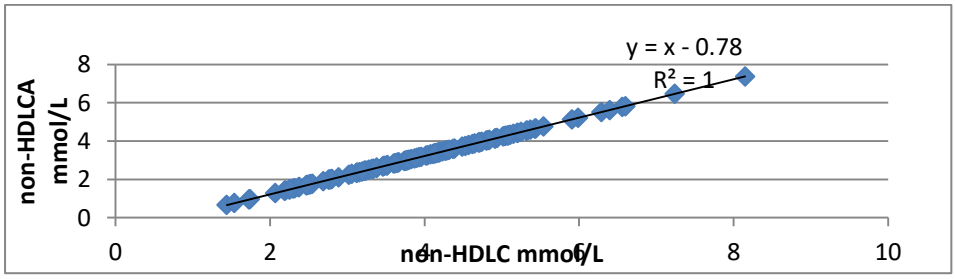

Figure 4: A linear regression analysis of the non-HDLCA and the non-HDLC methods

Figure 5 shows the association between cLDLC and non-HDLCA concentrations obtained from the study serum samples. The regression statistics obtained between the cLDLC (y) and the non-HDLCA (x) values were y = 0.8389x + 0.6291, with a correlation coefficient of 0.91 shows that the non-HDLCA and the cLDLC highly correlate in a linear manner.

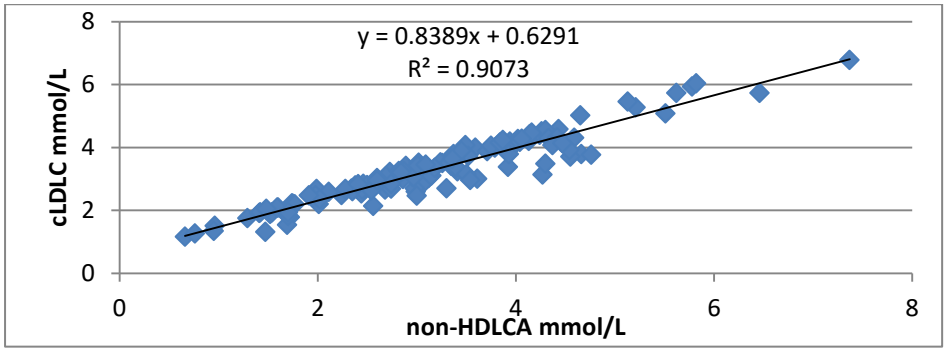

Figure 5: A linear regression analysis of the cLDLC and the non-HDLCA methods

Figure 6 shows the association between cLDLC and non-HDLC levels obtained from the study serum samples. The correlation statistics obtained between the cLDLC (y) and the non-HDLC (x) values were y = 0.8389x-0.0252, with a correlation coefficient of 0.91 shows that the cLDLC and the non-HDLC highly correlate in a linear manner, with a correlation coefficient of 0.91. The correlation coefficients of both non-HDLCA and non-HDLC with cLDLC is equal at 0.91

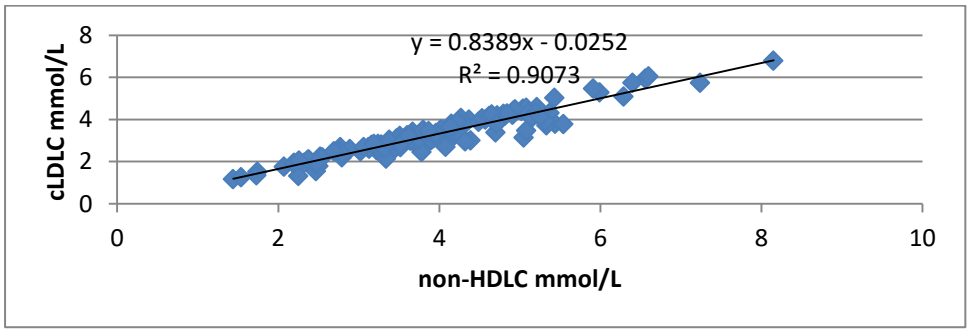

Figure 6: A linear regression analysis of the cLDLC and the non-HDLC

Figure 7 shows the correlation of the dLDLC and non-HDLCA concentrations obtained from the study serum samples. The correlation statistics obtained between the dLDLC (y) and the non-HDLCA (x) values where y = 1.1648x - 0.3446, with a correlation coefficient of 0.85 shows that the dLDLC and the non-HDLCA highly correlate in a linear manner, although the correlation is less compared to the higher correlation of the cLDLC and non-HDLCA/non-HDLC.

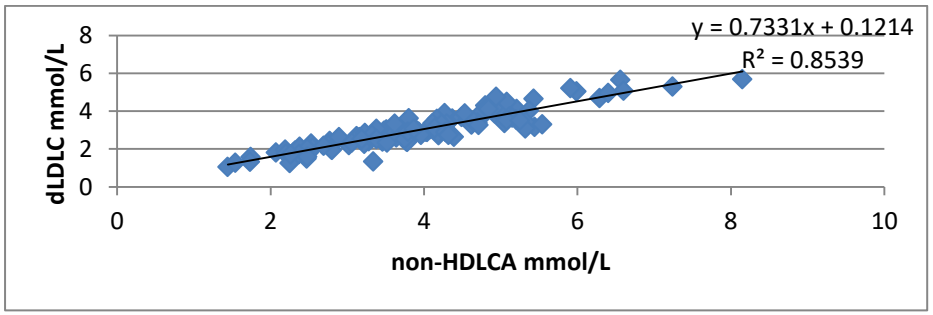

Figure 7: A linear regression analysis of the dLDLC and the non-HDLCA methods

Figure8 shows the correlation of the dLDLC and non-HDLC levels obtained from the study serum samples. The correlation statistics obtained between the dLDLC (y) and the non-HDLC values were y = 0.7331x + 0.1214, with a correlation coefficient of 0.85 shows that the dLDLC and the non-HDLC highly correlate in a linear manner, although the correlation is less compared to the higher correlation between the cLDLC and non-HDLC.

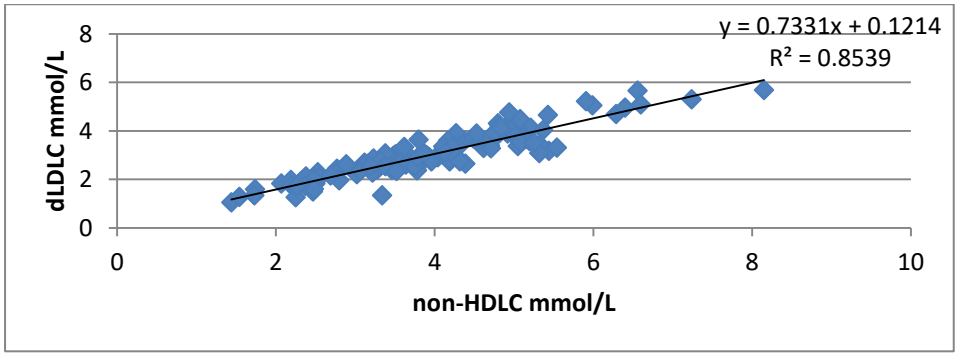

Figure 8: A linear regression analysis of the dLDLC and the non-HDLC

The cLDLC correlated highly with the dLDLC (r = 0.94), both the non-HDLCA and the non-HDLC correlated highly with the both the cLDLC and the dLDLC (r = 0.91, and r = 0.85 respectively), the non-HDLCA highly correlated with the non-HDLC (r = 1). The correlation results of non-HDLCA were the same with the non-HDLC. Other researchers indicate that the correlation coefficients of 0.89 and 0.80 for non-HDLCA and cLDLC, and non-HDLCA and dLDLC respectively, are strong correlations [42]. Therefore all the correlation coefficients obtained in this study were strong.

4.2 A comparison of cLDLC, dLDLC, non-HDLCA, and non-HDLC using LDLC/non-HDLCA and non-HDLC clinical cut-points based on NCEP CV risk category cut-off values

As shown in Table 5, cLDLC classified more serum samples in the higher CV risk categories than the dLDLC. The non-HDLC/non-HDLCA classified more patients in the higher CV risk categories compared to the dLDLC, but less compared to the cLDLC.

Table 5: Classification of sera into CV risk categories using the cLDLC, dLDLC, non-HDLC, and non-HDLCA.

CV risk category range (mmol/L)	cLDLC – n (%)	dLDLC – n (%)	Non-HDLC – n (%)	Non-HDLCA – n (%)	CV risk score
<2.59	28 (23)	40 (33)	39 (32)	39 (32)	Optimal
2.59 – 3.34	37 (31)	41 (34)	29(24)	30 (25)	Near/Above Optimal
3.35 – 4.13	31 (26)	26 (21)	26(21)	26 (21)	Borderline High
4.14 – 4.89	16 (13)	7 (6)	19(16)	18 (15)	High
>4.89	9 (7)	7 (6)	8(7)	8 (7)	Very High

n - Number of serum samples categorised into a risk category

4.2.1 CV risk classification results obtained after using dLDLC in place of cLDLC (Appendix 9.13)

In total 41 out of 121 (34%) serum samples had their CV risk categories changed upon using dLDLC in place of cLDLC. This corresponds to a clinical discordance of 34%. Thirty nine of the 41 of the CV risk category changes where in the form of a decrease by one CV risk category. One of the CV risk category changes was in the form of a decrease by two CV risk categories and one of the CV risk category changes wasin the form of an increase by one CV risk category upon using the dLDLC in place of the cLDLC. Most of the changes were reported in movement from H to BH; 9(22%), BH to N/AO; 16(39%) and N/AO to O; 12(29%).

4.2.2 CV risk classification results obtained after using non-HDLC in place of cLDLC (Appendix 9.14)

In total 39 out of 121 (32%) serum samples had their CV risk categories changed upon using non-HDLC in place of cLDLC. This corresponds to a clinical discordance of 32%. Twenty seven of the 39 (69%) of the CV risk category changes were in the form of a decrease by one CV risk category. Eleven (28%) of the CV risk category changes were in the form of an increase by one CV risk category and one (3%) of the CV risk category changes was in the form of an increase in two CV risk categories.

4.2.3 CV risk classification results obtained after using non-HDLC in place of dLDLC (Appendix 9.15)

In total 43 out of 121 (36%) serum samples had their CV risk categories changed upon using non-HDLC in place of dLDLC. This corresponds to a clinical discordance of 36%. Ten of the 43 (23%) CV risk category changes were in the form of a decrease by one CV risk category and three (7%) of the CV risk category changes were in the form of an increase by two CV risk categories. Thirty of the 43 CV risk category changes (70%) were in the form of an increase by one CV risk category.

4.2.4 CV risk classification results obtained after using non-HDLCA in place of non-HDLC (Appendix 9.16)

In total 1 out of 121 (0.8%) serum samples had their CV risk categories changed upon using non-HDLCA in place of non-HDLC The non-HDLCA and the non-HDLC showed a clinical concordance of 99.2% and a clinical discordance of only 0.8%. Only 1 sample had its CV risk category change in the form of a decrease by 1 CV risk category.

. This shows that the two calculations gave results that were similar and the difference was not statistically and medically different.

4.3 A comparison of cLDLC, dLDLC, non-HDLCA, and non-HDLC using the paired t test descriptive statistics (Appendices 9.25, 9.26, and 9.27)

Besides the linear correlation statistical analysis of the cLDLC, dLDLC, and the non-HDLCA and the CV risk classification of the patient sera using the LDLC and the non-HDLC/non-HDLCA; the paired t test statistical tool was also used in the analysis of the LDLC and the non-HDLCA serum results to establish whether the 3 methods of cLDLC, dLDLc, and the non-HDLC/non-HDLCA gave LDLC/non-HDLCA values which were significantly different. The paired t test calculations and the associated values are shown on Tables 6, 7, and 8. On all the comparisons; cLDLC with dLDLC (Table 6), cLDLC with non-HDLCA (Table 7), and dLDLC with non-HDLCA (Table 8), the results were significantly different.

Table 6: Descriptive statistics comparing the cLDLC and the dLDLC levels using the paired t test.

LDLC	n	mean	Std Deviation	Variance
cLDLC	121	3.2868	1.0773	1.1605
dLDLC	121	3.0158	0.9704	0.9416

n - Number of patient serum samples, p value <0.01

t = 10.8436, critical value = 2.617

The calculated t value of 10.8436 exceeds the critical value of 2.617. The mean difference of cLDLC and dLDLC at 0.271 was significantly different at p value < 0.01

Table 7: Descriptive statistics comparing the cLDLC and the non-HDLCA levels using the Paired t test.

	n	mean	Std Deviation	Variance
cLDLC	121	3.2868	1.0773	1.1605
Non-HDLCA	121	3.1685	1.2234	1.4968

n - Number of patient serum samples, p value <0.01

t = 3.3984, critical value = 2.617

The calculated t value of 3.3984 exceeds the critical value of 2.617. The mean difference of cLDLC and non-HDLCA at 0.118 was significantly different at p value < 0.01

Table 8: Descriptive statistics comparing the dLDLC and the non-HDLCA using the Paired t test.

	n	mean	Std Deviation	Variance
dLDLC	121	3.0158	0.9704	0.9416
Non-HDLCA	121	3.1685	1.2234	1.4968

n - Number of patient serum samples, p value <0.01

t = -3.3987, critical value = 2.617

The calculated t value of 3.3987 exceeds the critical value of 2.617. The mean difference of dLDLC and non-HDLCA at 0.153 was significantly different at p value < 0.01

4.4 A comparison of HDLC/LDLC lipid ratios with LDLC (Appendices 9.17, 9.18, 9.19, and 9.20)

A general overestimation of CV risk estimation on using HDLC/LDLC ratios as compared to LDLC was observed from Table 9. Also, an overestimation of CV risk when a cLDLC based ratio was used in place of dLDLC was observed.

Table 9: A comparison of the CV risk estimation when the HDLC/LDLC lipid ratios were used in place of the LDLC methods

Lipid Based Comparison	Total CV risk category changes n (%)	Nature of CV risk category changes	
		R to NR n (%)	NR to R n (%)
HDLC/cLDLC in place of cLDLC	30 (24.8%)	24 (80.0%)	6 (20.0%)
HDLC/dLDLC in place of cLDLC	39 (32.2%)	34 (87.2%)	5 (12.8%)
HDLC/cLDLC in place of dLDLC	30 (24.8%)	20 (66.7%)	10 (33.3%)
HDLC/dLDLC in place of dLDLC	34 (28.1%)	26 (76.5%)	8 (23.5%)

In total 30 out of 121 (24.8%) serum samples had their CV risk categories changed upon using HDLC/cLDLC in place of cLDLC. This corresponds to a clinical discordance of 24.8%. In total 39 out of 121 (32.2%) serum samples had their CV risk categories changed upon using HDLC/dLDLC in place of cLDLC. This corresponds to a clinical discordance of 32.2%. In total 34 out of 121 (28.1%) serum samples had their CV risk categories changed upon using HDLC/dLDLC in place of dLDLC. This corresponds to a clinical discordance of

28.1%. When the dLDLC was replaced with the HDLC/cLDC, 30 serum samples out of the total 121 (24.8%) had their CV risk categories changed. The CV risk category changes correspond to a clinical discordance of 24.8%.

4.5 A comparison of the CV risk estimation on using the HDLC/dLDLC and HDLC/cLDLC (Appendices 9.21 and 9.28)

Figure 9 below shows the correlation of the HDLC/dLDLC and HDLC/cLDLC levels obtained from the study serum samples. The regression statistics obtained between the HDLC/dLDLC (y) and the HDLC/cLDLC (x) values were y = 0.9453x+0.0574, with a correlation coefficient of 0.93 shows that the HDLC/dLDLC and HDLC/cLDLC highly correlate in a linear manner.

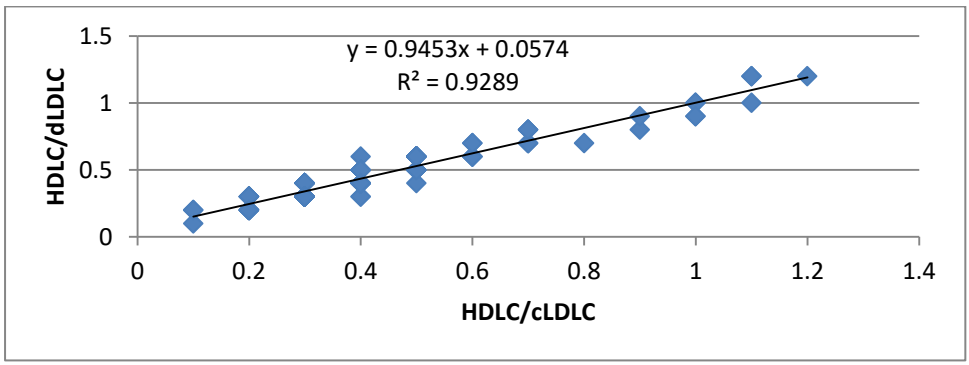

Figure 9: A linear regression analysis of the HDLC/cLDLC and the HDLC/dLDLC values

The descriptive statistics comparing the HDLC/dLDLC and the HDLC/cLDLC using a Paired t test are presented in Table 10.

Table 10: Descriptive statistics comparing the HDLC/dLDLC and the HDLC/cLDLC using a Paired t Test.

Lipid Ratio	n	mean	Std Deviation	Variance
HDLC/dLDLC	121	0.4645	0.2249	0.0506
HDLC/cLDLC	121	0.4306	0.2293	0.0526

n - Number of patient serum samples, p value <0.01

t = -6.0804, critical value = 2.617

The calculated t value of 6.0804 exceeds the critical value 2.617. Therefore the means of HDLC/dLDLC and the HDLC/cLDLC ratios at 0.4645 and 0.4306 respectively and the mean difference at 0.0339 are significantly different at p value <0.01).

Nine out of 121 (7%) serum samples had their CV risk categories changed (from Risk to Low Risk) when HDLC/dLDLC was used in place of the HDLC/cLDLC. A general overestimation of the CV risk when the HDLC/cLDLC ratio was used in place of the HDLC/dLDLC was observed over the 1.16 – 5.94 mmol/L LDLC concentration range.

4.6 A comparison of LDLC and non-HDLC with sdLDLC (Appendices 9.22, 9.23, and 9.24 and Table 11)

In total, 12 out of 121 (9.9%) serum samples had their CV risk categories changed upon using sdLDLC in place of cLDLC. This corresponds to a clinical discordance of 9.9%. The majority of CV risk category changes, 10 (83.3%) were a movement from Low Risk to Risk category. Twenty one out of 121 (17.4%) serum samples had their CV risk categories changed

upon using sdLDLC in place of dLDLC. This corresponds to a clinical discordance of 17.4%. All the CV risk category changes were a movement from LR to R category. Twenty out of 121 (16.5%) serum samples had their CV risk categories changed upon using sdLDLC in place of non-HDLC. This corresponds to a clinical discordance of 16.5%. All the CV risk category changes were a movement from LR to R category.

Table 11: A comparison of the CV risk estimation on using the sdLDLC in place of the cLDLC, dLDLC, and non-HDLC

Lipid Based Comparison	Total CV risk category changes n (%)	Nature of CV risk category changes	
		R to LR n (%)	LR to R n (%)
sdLDLC in place of cLDLC	12(9.9%)	2(16.7%)	10(83.3%)
sdLDLC in place of dLDLC	21(17.4%)	0(0%)	21(100%)
sdLDLC used in place of non-HDLC	20(16.5%)	0(0%)	20(100%)

4.7 A comparison of lipid based CV risk biomarkers in the determination of CV risk in patients' sera.

The categorisation of sera lipid measurements into Low Risk (also known as normal) and Risk (Table 12) is based on the Lipid and Atherosclerosis Society of Southern Africa and Ampath Laboratories guidelines in moderate risk cases which classifies the optimal risk category into Low Risk and all the other risk categories into the Risk category [6].

Subsequently, the optimal CV risk category only was classified into Low Risk category while near/above optimal, the borderline high, the high, and the very high CV risk categories were classified into the Risk category.

Table 12: Classification of sera into Low risk and Risk categories using the cLDLC, dLDLC, non-HDLC, sdLDLC, HDLC/cLDLC, and HDLC/dLDLC.

	Parameter	Frequency n (%)	
		Low Risk	Risk
1	cLDLC	28 (23.1%)	93 (76.9%)
2	dLDLC	40 (33.1%)	81 (66.9%)
3	non-HDLC	39 (32.2%)	82 (67.8%)
4	sdLDLC	20 (16.5%)	101 (83.5%)
5	HDLC/cLDLC	49 (40.5%)	72 (59.5%)
6	HDLC/dLDLC	58 (47.9%)	63 (52.1%)

5. DISCUSSION

5.1 Comparison of the cLDLC, the dLDLC, and the non-HDLCA/non-HDLC methods

5.1.1 A regression analysis comparison of cLDLC, dLDLC, and non-HDLCA/non-HDLC

The lipid based measurements of cLDLC, dLDLC, and non-HDLCA/non-HDLC showed high correlation in a linear manner with each other. The correlations of cLDLC with the dLDLC (Figure 3), non-HDLCA/non-HDLC with the cLDLC (Figures 5 and 6), and the non-HDLCA/non-HDLC with the dLDLC (Figures 7 and 8) were 0.94, 0.91, and 0.85 respectively. The high correlations suggest that the methods had a high degree of agreement and therefore can be used interchangeably in the CV risk prediction and treatment assessment. These findings concur with findings by other researchers [13,21,37,39]. Mora et al [21] established that the Friedewald cLDLC and the Roche dLDLC correlated highly with a correlation coefficient of 0.97. These high correlations demonstrated that there was no advantage for using the Roche Diagnostics dLDLC method in place of the Friedewald method [21]. Some researchers established that the Roche dLDLC method performed no better than the Friedewald cLDLC [39]. Other researchers comparing the cLDLC and a direct homogeneous method other than the Roche also found a good linear correlation between the two methods. Also, in another study, the dLDLC was interpreted to be as good as the cLDLC. Taking into account the extra cost introduced by the use of dLDLC the cLDLC was recommended [13].

Few studies have been carried out comparing the non-HDLC with the cLDLC and the dLDLC methods [45]. Baruch et al [45] established that the adjusted non-HDLC (non-HDLCA) correlated highly in a linear manner with the cLDLC and the dLDLC with correlation coefficients of 0.89 and 0.80 respectively [45]. Findings from this study agreed

with Baruch et al findings on the correlation of non-HDLCA with cLDLC and dLDLC. This study's correlation coefficients of non-HDLCA with cLDLC (Figure 6) and non-HDLCA with dLDLC (Figure 7) were also high and in a linear manner at 0.91 and 0.85 respectively. The high correlations from this study and from Baruch et al suggest that the non-HDLCA/non-HDLC and the LDLC methods have a high degree of agreement and therefore can be used interchangeably in the CV risk prediction and treatment assessment if the correlation coefficient is to be used as an indicator of how close the results are to each other [13].

5.1.2 A comparison of cLDLC, dLDLC, non-HDLCA, and non-HDLC using LDLC/non-HDLCA and non-HDLC clinical cut-points based on NCEP CV risk categories.

On comparing the cLDLC, dLDLC, and non-HDLCA and non-HDLC using the LDLC and non-HDLC NCEP CV risk category cut-off values, the three tests showed a lot of clinical discordance with each other, suggesting that they cannot be used interchangeably in the CV risk prediction and treatment assessment. This study found 41 (34%) CV risk category changes when the dLDLC was used in place of the cLDLC. Furthermore, an overwhelming decrease in LDLC estimation which did not result in the change in the CV risk category was observed when the dLDLC was used in place of the cLDLC. The dLDLC classified sera into lower CV risk categories than the cLDLC on the basis of cut-off values set by NCEP as shown in Table 5. At 34%, the CV risk category changes were significant. This corresponds to a clinical discordance of 34%. The method dependent variation of LDLC values results in classification of individuals' serum samples into different CV risk prediction and treatment categories as shown in Table 5. The high clinical discordance was observed despite the high correlations among the three tests. This concurs with the research findings of Baruch et al [45].

In this study, the adjusting of non-HDLC to non-HDLCA did not improve the non-HDLC component on regression and CV risk assessment. The non-HDLC did not provide the same clinical information to either cLDLC or dLDLC in individual patients. This observation is parallel to what Baruch et al [45] observed. This study indicates a variation in the CV risk classification of patient sera in CV risk categories when non-HDLC is used in place of LDLC. Upon use of non-HDLC in place of cLDLC, 39 (32%) out of 121 serum samples had their CV risk categories changed. This corresponds to a clinical discordance of 32%. The majority of these CV risk category changes were by a lowering by one CV risk category. These CV risk category changes were significant. Upon use of non-HDLC in place of dLDLC, 43 (36%) out of 121 serum samples had their CV risk categories changed. This corresponds to a clinical discordance of 36%. The majority of these CV risk category changes were by an increase by one CV risk category. These CV risk category changes were significant. Baruch et al [45] had similar findings.

The non-HDLC overestimated CV risk compared to dLDLC and underestimated CV risk compared to cLDLC. The CV risk differences were statistically and medically significantly different. The non-HDLC and the dLDLC , both of which are the recommended alternative lipid measurements when cLDLC is inaccurate, appear to be the most divergent of the lipid pairs as shown by the highest clinical discordance of 36% compared to the cLDLC and dLDLC, and non-HDLC and cLDLC lipid pairs which have lower discordances of 34% and 32% respectively. Baruch et al [45] had similar findings. The non-HDLC and the dLDLC must not be used interchangeably since the two lipid measurements are not providing the same diagnostic information in serum samples. Non-HDLC is assumed to be a better predictor of CV risk since it is a measure of total atherogenicity [15]. Given that the non-HDLC represents the total atherogenesis making it a better predictor of CV risk than LDLC

values [16,17,46], and that dLDLC is a better measure of LDLC associated CV risk than cLDLC, it is better to follow the non-HDLC and dLDLC trend than the cLDLC trend.

5.1.3 Paired t test analysis of cLDLC, dLDLC, and non-HDLCA/non-HDLC

The paired t test descriptive statistics used to compare the cLDLC, dLDLC, and non-HDLCA/non-HDLC showed that these lipid based measurements gave significantly different values. This indicates that the three lipid measurements cannot be used interchangeably in the CV risk prediction and treatment assessment. The statistical data calculated using the paired t test showed that the means of the cLDLC and the dLDLC (Table 6), the means of the non-HDLCA and the cLDLC (Table 7), and the the means of the non-HDLCA and the dLDLC (Table 8) were significantly different at $p < 0.01$. This study indicates an average 0.271 mmol/L analytical error (%) in LDLC estimation when the cLDLC was used in place of the dLDLC. The comparison of cLDLC and dLDLC analytical error in this study is comparable with the one established by Mauricio et al [2].

The use of the lipid profile in the initial dyslipidaemia screening allows the investigation of other dyslipidaemia elements like hypertriglyceridaemia and low HDLC in addition to LDLC associated dyslipidaemia. Also, the use of the lipid profile allows the use of lipid calculations which enrich the diagnosis of dyslipidaemia and categorisation of the CV risk. In the event of using the dLDLC alone, only the dyslipidaemia associated with LDLC is available for dyslipidaemia, CV risk assessment, and treatment monitoring. Most dyslipidaemia cases are true hypercholesterolaemias in the form of TC elevations due to LDLC elevations. Therefore, LDLC will be sufficient for dyslipidaemia diagnosis, CV risk establishment, and treatment assessment in most cases. At intervals, the doctors will need a full lipid profile with TC, TG, HDLC, LDLC, and since the common lipid lowering drugs not only reduce LDLC, but also reduces TGs and increase HDLC warranting their assaying [37].

The reagent cost advantage favours cLDLC over dLDLC method in the lipid profile. The use of the lipid profile with cLDLC looks cheaper than the use of the lipid profile with direct homogeneous methods for example the Roche Direct homogeneous method.

However, cost-effectiveness must consider the direct and indirect costs arising from measurement (analytical) errors leading to inappropriate treatment decisions. An objective assessment of such costs is likely to support the direct measurement, but it will require more studies, including consideration of outcomes [16].

The accuracy of the direct methods varies and the interpretation of LDLC results is method based. The results from this study are applicable to the direct LDLC based on the Roche Diagnostic direct homogeneous method only. Other direct methods are likely to give different results [11,16,32]. For that reason, results from this study may not be extended to other methods, other than the Roche Diagnostic direct homogeneous method with respect to the dLDLC [11,16,32].

5.1.4 Comparison of the cLDLC, the dLDLC, and the non-HDLCA/non-HDLC methods using the regression analysis, NCEP CV risk categories, and the paired t test

In this study the use of regression correlation analysis was found to be misleading in the comparison of LDLC and non-HDLC methods in lipid based CV risk prediction and treatment assessment. The regression correlation analysis indicated that the dLDLC, cLDLC, and the non-HDLCA/non-HDLC gave results that were not significantly different. The high correlations suggest that the methods had a high degree of agreement and therefore can be used interchangeably in the CV risk prediction and treatment assessment while the CV risk categories classification and the paired t test indicated that the dLDLC, dLDLC, and the non-HDLCA gave results that were clinically and statistically significantly different. The

disagreement between the clinical discordance and the correlation arises from the correlation coefficient being a summary statistic rather than a statistic that defines relationships in individual patients [45].

The clinical discordance is in line with the paired t test calculated significance values between the cLDLC, dLDLC, and non-HDLCA/non-HDLC lipid measurements. In non-HDLC, the clinical discordance and the paired t test findings are in line with theory which acknowledges that LDLC and non-HDLC measure different entities of atherogenicity. The clinical discordance and the paired t test findings are in line with theory which acknowledges the unacceptable and significantly different results coming from the cLDLC method which has a high total analytical error (>12%) and is known to overestimate the LDLC levels [1,2], and the dLDLC that has an acceptable analytical error (<12%) [14].

This study proves that the cLDLC overestimation of LDLC and the differences in the cLDLC and the dLDLC results were clinically and statistically significant when the CV risk classification and the paired t test methods respectively, were used in cases with TGs <4.52mmol/L. These findings are in line with other research findings [1,16,21,29], and contradicts findings by other researchers [31]. This study indicates that with TG levels < 4.52 mmol/L, the cLDLC overestimates the serum LDLC thereby affecting clinical decision making [16] as illustrated in Table 5. The findings of this current study justify statistically and clinically that the Friedewald method (cLDLC) is not good for estimation of serum LDL concentrations in serum samples with TG <4.52 mmol/L [16]. This is in contrast to established guidelines like the NCEP ATP III, reports from other studies, and some expert panels who have endorsed that the cLDLC overestimation of LDLC is insignificant at TGs levels <4.52 mmol/L. In routine clinical practice, the Friedewald method is deemed

acceptable with TGs <4.52mmol/L [1]. According to NCEP guidelines cLDLC is not reliable for LDLC estimation when TG >4.52mmol/l [14]. Currently serum with elevated TG levels ≥ 4.52 mmol/L are considered a limiting factor for the application of the Friedewald method (cLDLC) due to the underestimation of LDLC levels in those specimens [14]. Given that the Friedewald method is not recommended in cases with TG levels ≥4.52 mmol/L [13] (where the direct homogeneous methods are recommended) and that this study establishes that the Friedewald method is not good for TGs levels <4.52 mmol/L, concurring with other research findings [16,29], this study therefore found the Roche Diagnostics direct homogeneous method (dLDLC) to be a better method than the Friedewald method in LDLC estimation.

5.2 A comparison of the HDLC/LDLC with the LDLC

Studies have confirmed that the HDLC/LDLC ratio is excellent CV risk prediction and LLDT monitoring. The changes in the HDLC/LDLC ratios have been shown to better assess the CV risk compared with the absolute levels like LDLC [15]. In this study, the HDLC/LDLC ratios (HDLC/cLDLC and HDLC/dLDLC) classified more serum samples into the Risk category compared to LDLC (Table 9). The HDLC/cLDLC and the HDLC/dLDLC classified 72 and 63 serum samples into the Risk category respectively. In comparison cLDLC and dLDLC classified only 56 and 40 samples into the Risk category. The increase in the number of the cases classified under Risk is likely to reflect improved sensitivity of the lipid ratios as compared to the LDLC levels. The HDLC and the LDLC represent the particles involved in the transport of cholesterol in the forward cholesterol transport and the reverse cholesterol transport respectively (antagonising cholesterol transport) [20,21,29]. The lipid ratios therefore represent a more comprehensive measure of atherogenicity [15].

Given that dLDLC is a better measure of LDLC associated CV risk than cLDLC [1,2,14], it is advisable to follow the HDLC/dLDLC based values instead of the CV risk overestimating cLDLC values. HDLC/dLDLC classified 63 sera into the Risk category while the cLDLC classified 56 sera into the Risk category.

5.3 A comparison of HDLC/dLDLC with HDLC/cLDLC

In this study HDLC/cLDLC classified more serum samples under the Risk category than the HDLC/dLDLC. The correlation of the two ratios was high at 0.93 (Figure 9). The paired t test showed that the difference of the results between the two ratios were statistically significant (Table 10). However only 7% of the serum samples had their CV risk categories changed when the HDLC/dLDLC was used in place of the HDLC/cLDLC. This corresponds to a clinical discordance of 7% and a clinical concordance of 93%.Therefore the two methods can be used interchangeably since they give the same clinical results in almost all the serum samples. Given that dLDLC is a better measure of LDLC associated CV risk than cLDLC [1,2,14], it is advisable to follow the HDLC/dLDLC based values instead of the HDLC/cLDLC.

5.4 A comparison of LDLC and non-HDLC with sdLDLC

The sdLDLC categorised more sera into the Risk category relative to the LDLC and non-HDLC biomarkers. On using sdLDLC in place of the dLDLC and the non-HDLC no serum sample had its CV risk category changed from Risk to Low Risk while 21 (17.4%) and 20 (17%) serum samples had their CV risk category changed from Low Risk to Risk respectively. On using the sdLDLC in place of the cLDLC only 2 (1.7%) of the serum samples had their CV risk categories changed from Risk to Low Risk while 12 (9.9%) serum samples had their CV risk category changed from Low Risk to Risk . Therefore the sdLDLC

biomarker gave a diagnosis of CV risk more frequently compared to the cLDLC, dLDLC and non-HDLC. In this study, the correlation coefficients of the sdLDLC with the cLDLC, dLDLC, non-HDLC, HDLC/cLDLC, and the HDLC/dLDLC were 0.59, 0.67, 0.82, 0.39, and 0.43 respectively. The findings are consistent with the reports of a study by Srisawasdi et al whose results showed that the non-HDLC levels had the strongest correlation relationship with the sdLDLC levels (r=0.87) compared with other lipid measurements [18]. On the CV risk category changes, the sdLDLC classification of risk followed the one of cLDLC the closest compared to those of dLDLC and non-HDLC. More evaluation is needed to establish the entities being measured by the different lipid biomarkers.

The thorough research and wide use of dLDLC is likely to be masked by the evolution of dyslipidaemia that has increased the prevalence of the atypical sdLDLC due to the global increase of metS. The LDLC methods, including the dLDLC method miss the sdLDLC variant of LDLC dyslipidaemia, resulting in poor CV risk prediction. Therefore biomarker targets like non-HDLC and sdLDLC are more likely to be more significant in the CV risk prediction and treatment assessment in the near future. Given that non-HDLC adds no cost on the routine lipid profile while the sdLDLC calculation adds an additional cost with the additional dLDLC measurement [43,44], the non-HDLC becomes the cheaper and also likely more sensitive CV risk predictor and treatment assessment tool. The non-HDLC is likely to replace or join cLDLC as the primary target of CV risk prediction and treatment assessment.

5.5 A comparison of lipid based CV risk biomarkers in the determination of CV risk in serum samples.

Classification of sera into Low Risk and Risk categories using the cLDLC, dLDLC, non-HDLC, sdLDLC, HDLC/cLDLC, and HDLC/dLDLC was done. The usage of different lipid based methods resulted in the classification of sera into different CV risk categories. The cLDLC, dLDLC, non-HDLC, sdLDLC, HDLC/cLDLC, and HDLC/dLDLC classified 93, 81, 82, 101, 72, and 63 serum samples into the Risk category respectively. The differing categories will: leave some patients out of treatment when they are actually candidates for treatment; result in the unnecessary treatment of CV risk negative cases; put some patients into incorrect risk categories resulting in unnecessary treatment, unnecessarily aggressive treatment, wrong treatment, or suboptimal treatment which will result in unnecessary side effects like hepatotoxicity, unnecessary expenses, and treatment failure. The overall effect of incorrect classification of patients in the CV risk categories is the increase in morbidity and mortality and the increase in the cost of healthcare delivery [10].

5.6 Suggested Lipid profile algorithm for lipid based CV risk assessment and treatment monitoring.

The proposed lipid profiling is based on the study findings and literature review

a) Initial screening/diagnosis – a full lipid profile with a dLDLC (TC, TG, HDLC, and dLDLC), plus lipid based calculations of non-HDLC, sdLDLC, and HDLC/dLDLC
b) Treatment assessment – dLDLC only
c) Determined intervals - a lipid profile with a dLDLC (TC, TG, HDLC, and dLDLC), plus lipid based calculations of non-HDLC, sdLDLC, and HDLC/dLDLC

6. CONCLUSION

When using CV risk classification/clinical discordance and paired t test, the overestimation of CV risk by the cLDLC compared to the dLDLC has been confirmed in this study. Given that the overestimation of LDLC in the Friedewald method is well established in literature, the overestimation of LDLC by the Friedewald method relative to the Roche Diagnostics direct homogeneous method has been observed in this study, and that the national guidelines and the expert panels use LDLC as a primary target in CV risk estimation and treatment assessment, the dLDLC must replace the cLDLC in CV risk prediction and treatment assessment. It is advisable for laboratories in the same country to use the same direct homogeneous method. It has been noted that the sample of choice in dyslipidaemia CV risk assessment is a random blood sample. Also, the five lipid CV risk assessment methods classified patients into different CV risk categories and hence into separate intervention categories when the clinical discordance and the paired t test were used. Therefore the different lipid CV risk estimation methods cannot be used interchangeably. The regression correlation analysis statistical tool must not be used in comparing CV risk estimation methods since it was found to be misleading in this study. The differences in the CV risk estimation from the different lipid CV risk estimation methods may reflect incremental or additional CV risk, a separate entity of CV risk not measured by the other method(s), or the same entity of CV risk measured by other methods with varying sensitivities. There is urgent need to re-evaluate the non-HDLC as a CV risk predictor and treatment assessment target. The usefulness of the LDLC in CV risk estimation need to be re-evaluated and the usefulness of other lipid based measurements, other than LDLC, need to be verified. This current research found sdLDLC as the best marker for assessment of CVD as it categorised more patients into the risk category than any of the other methods evaluated. However, since national guidelines and expert panels have not yet endorsed the sdLDLC as a target for the

diagnosis and treatment monitoring in CV risk assessment, sdLDLC will not be recommended in this study as the target for CV risk assessment and treatment monitoring. It potents as a good marker for risk assessment but is not yet widely used as there are no guidelines for standardising its application in patients. More research is needed on sdLDLC and guidelines have to be published for it to be adopted as a risk marker for CVD in clinical practice. The findings in this research including the findings that sdLDLC detected more risk than other CV risk estimation and assessment tools will contribute to the knowledge on the topic. These findings and findings elsewhere will contribute towards the formulation of the next generation of guidelines. It is important that validated algorithms with best specificities and sensitivities are put in place for CV risk estimation and treatment assessment. The regression correlation statistical tool must not be used in lipid biomarkers methods comparison. The research finding will be disseminated through electronic publications.

7. RECOMMENDATIONS

a) Laboratories in the same country or region are recommended to use the same LDLC method (preferably the dLDLC) for CV risk estimation, treatment initiation, and treatment monitoring. LDLC method variation will cause method dependent (not pathological/physiological) serum LDLC level variation which will give false results. This will result in incorrect lipid based CV risk estimation and management.

b) This research must be repeated on a wider scale preferably with the inclusion of the Beta Quantification reference method.

c) The dLDLC must replace the cLDLC for initiating and monitoring therapy in CVD.

d) More studies using large samples taken from different ethnic and geographic populations so as to include varying genetic predispositions, diets, seasonal variations and other factors and preferably compared with the Beta Quantification reference method is needed to verify findings from this study.

e) More research is needed to establish the best lipid based profile(s) from these affordable and available lipid based tests to enhance the diagnosis and assessment of CV risk. Establishment of the best lipid based profile(s) will enrich the dyslipidaemia CV risk prediction and treatment assessment without increasing the cost of the AACVD diagnosis and assessment.

f) More research is needed with other non-routine lipid based tests like the NMR and VAP, and also with non-lipid based tests like inflammatory biomarkers to enhance the diagnosis and assessment of CV risk.

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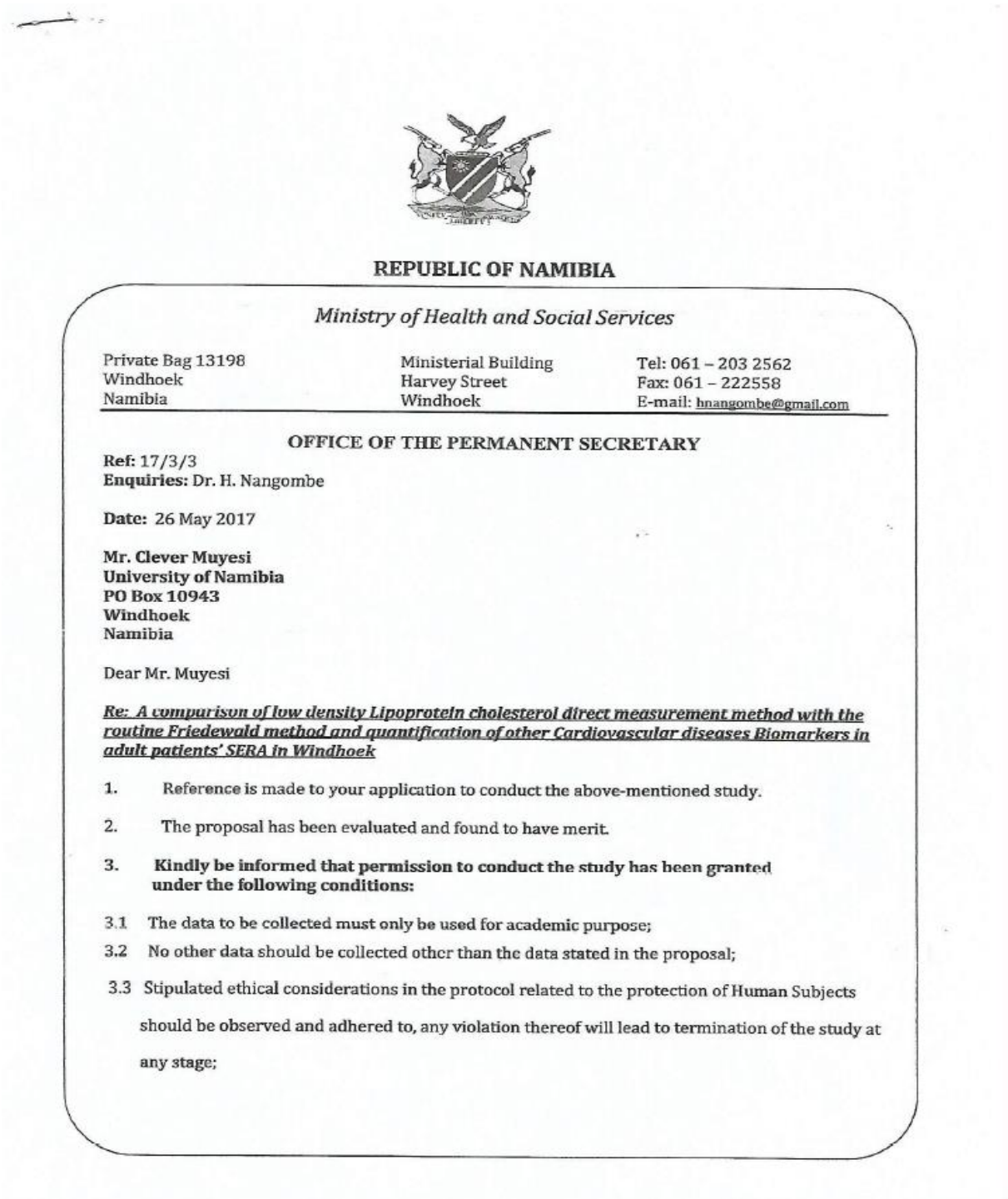
9. APPENDICES

Appendix 9.1 Ministry of Health and Social Services research approval letter.



REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

Private Bag 13198
Windhoek
Namibia

Ministerial Building
Harvey Street
Windhoek

Tel: 061 – 203 2562
Fax: 061 – 222558
E-mail: hnangombe@gmail.com

OFFICE OF THE PERMANENT SECRETARY

Ref: 17/3/3
Enquiries: Dr. H. Nangombe

Date: 26 May 2017

Mr. Clever Muyesi
University of Namibia
PO Box 10943
Windhoek
Namibia

Dear Mr. Muyesi

Re: A comparison of low density Lipoprotein cholesterol direct measurement method with the routine Friedewald method and quantification of other Cardiovascular diseases Biomarkers in adult patients' SERA in Windhoek

1. Reference is made to your application to conduct the above-mentioned study.

2. The proposal has been evaluated and found to have merit.

3. **Kindly be informed that permission to conduct the study has been granted under the following conditions:**

3.1 The data to be collected must only be used for academic purpose;

3.2 No other data should be collected other than the data stated in the proposal;

3.3 Stipulated ethical considerations in the protocol related to the protection of Human Subjects should be observed and adhered to, any violation thereof will lead to termination of the study at any stage;

Appendix 9.1 (continued) Ministry of Health and Social Services research approval letter.

3.4 A quarterly report to be submitted to the Ministry's Research Unit;

3.5 Preliminary findings to be submitted upon completion of the study;

3.6 Final report to be submitted upon completion of the study;

3.7 Separate permission should be sought from the Ministry for the publication of the findings.

Yours sincerely,

Andreas Mwoombola (Dr)
Permanent Secretary

"Health for All"

Appendix 9.2 University of Namibia Research support letter

University of Namibia, Private Bag 13301, Windhoek, Namibia
340 Mandume Ndemufayo Avenue, Pioneerspark
☎ +264 61 206 3111, URL - http://www.unam.edu.na

UNAM
UNIVERSITY OF NAMIBIA

To Whom It May Concern,

Mr Clever Muyezi is a Master of Science student in Biochemistry at the University of Namibia – UNAM (Student Registration Number 201145618). He is currently conducting a research on - A COMPARISON OF LOW DENSITY LIPOPROTEIN CHOLESTEROL DIRECT MEASUREMENT METHOD WITH THE ROUTINE FRIEDEWALD METHOD AND QUANTIFICATION OF OTHER CARDIOVASCULAR DISEASES BIOMARKERS IN ADULT PATIENTS' SERA IN WINDHOEK.

Please support him in his research.

Mr Muyezi's study will be carried out at CLS Pathology Xpress cc and Medical Diagnostic Laboratory Pty Ltd laboratories which are all certified by the MoHSS to handle human samples and information. All patient information and the subsequently generated results will be treated with confidentiality. The supervisors for this study are:

Dr Petrina Kapewangolo (Main Supervisor: Department of Chemistry & Biochemistry, University of Namibia Tel: +264 61 206 3384, Fax: +264 61 206 3791 pkapewangolo@unam.na) and Mr Munyaradzi Mukesi (Co-supervisor: Department of Biomedical Sciences, Namibia University of Science and Technology Tel: +264 61 207 2970, Fax: +264 61 207 9970 mukesi@yahoo.co.uk, mmukesi@nust.na,

Should there be any queries, please feel free to contact the supervisors.

UNAM
UNIVERSITY OF NAMIBIA
Faculty of Science
Office of the Dean
2017 -02- 17
Signature:.....

Sincerely,

Petrina Kapewangolo (PhD)
Senior Lecturer: Chemistry & Biochemistry department
Faculty of Science, University of Namibia
pkapewangolo@unam.na
+264 61 206 3384

Appendix 9.3 University of Namibia Ethical Clearance Certificate

UNAM
UNIVERSITY OF NAMIBIA

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: FOS/137/2016 **Date: 5 December, 2016**

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

Title of Project: a Comparison of Low Density LipoProtein Cholestrol Direct Measurement Method With the Routine Friedewald Method and Quantification of Other Cardiovascular Diseases Biomarkers in Adult Patients' Sera in Windhoek

Nature/Level of Project: Masters

Researcher: C. Muyezi

Student Number: 201145618

Faculty: Faculty of Science

Supervisors: Dr. P. Kapewangolo (Main) Mr. M. Mukesi (Co)

Take note of the following:

(a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the UREC. An application to make amendments may be necessary.
(b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the UREC.
(c) The Principal Researcher must report issues of ethical compliance to the UREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by UREC.
(d) The UREC retains the right to:

(i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
(ii) Request for an ethical compliance report at any point during the course of the research.

UREC wishes you the best in your research.

Prof. P. Odonkor: UREC Chairperson

Ms. P. Claassen: UREC Secretary

Appendix 9.4

CLS Pathology Xpress Ministry of Health and Social Services licence

License No: Khomas 906

Republic of Namibia

Ministry of Health and Social Services

Certificate

This is to certify that

CLS PATHOLOGY EXPRESS

physical address

Erf F2014/02715, Unit 1009, Sam Nujoma Drive, Westcare Medical Centre, Windhoek

is licensed in terms of

Section 31 of the Hospitals and Health Facilities Act, 1994
(Act 36 of 1994)

for the period

21–Jul- 18 to 20–Jul- 19

as a

Medical Laboratory

PERMANENT SECRETARY
MINISTRY OF HEALTH & SOCIAL SERVICES

PERMANENT SE

19- July 2018

DATE

Appendix 9.5 Serum sample collection worksheet

NamPath Laboratory Worksheet

Researcher – Clever Muyezi

Research topic - A COMPARISON OF LOW DENSITY LIPOPROTEIN CHOLESTEROL DIRECT MEASUREMENT METHOD WITH THE ROUTINE FRIEDEWALD METHOD AND QUANTIFICATION OF OTHER LIPIDIC CARDIOVASCULAR DISEASE BIOMARKERS IN ADULT PATIENTS' SERA IN WINDHOEK

Sample collection form

Laboratory /Doctor NAMPATH LABORATORY

Seq no.	Lab no.	Sample collection date	Sample collection time	Serum separation time	Serum aliquoting and refredgiration date	Serum aliquoting and refredgiration time	Gender/ Age	Initi
1 R	NPW0022267	20/4/18	9:55	10:15	20/4/18 10:25	10:25	Male 49	LM
2 R	NPW0022307	24/4/18	08h10	09h15	24/4/18	09h25	M 62	MC
3 R	NPW0022308	24/04/18	08h00	09h15	24/4/18	09h25	M 40	M
4 F	NPW0022318	24/04/18	09h00	09h15	24/4/18	09h25	F 55	[illegible]
5 R	NPW0022320	23/4/18	12h35	09h30	24/4/18	09h40	F 27	[illegible]
6 R	NPW0022322	23/4/18	11h30	09h30	24/4/18	09h40	M 64	[illegible]
7 R	NPW0022323	23/4/18	09h40	09h30	24/4/18	09h40	F 63	[illegible]
8 R	NPW0022327	23/4/18	12h30	10h00	24/4/18	10h10	F 37	[illegible]
9 R	NPW0022328	23/4/18	12h00	10h00	24/4/18	10h10	M 48	[illegible]
10 R	NPW0022330	23/4/18	13h30	10h00	24/4/18	10h10	M 39	[illegible]

Fasting/Non-fasting lipogram sample collection and handling guidelines

1) Draw sufficient volume of fasting or non-fasting venous whole blood into labelled yellow top SST gel specimen tube(s).
2) Allow the blood to clot at room temperature for 30 to 60 minutes.
3) After clot formation, centrifuge the specimen at 3000g for 10 minutes.
4) Transfer the serum into 2 labelled aliquot or transport tube immediately after centrifugation or after analysing on the analyser(s).
5) Place the serum aliquots in a 2 8 degree celcius holding freezer immediately after aliquoting.
6) The serum aliquots will be picked by the researcher at the end of the working day.

Appendix 9.6 CLS Cobas c111 Instrument Validation

Appendix 9.6.1 Thistle EQA

Total cholesterol

	Analyte – Total cholesterol, acceptable CV = 13			
	Thistle QA results (mmol/L)	CLS results (mmol/L)	% Deviation	Validation outcome
1	6.82	7.07	3.54	pass
2	4.16	4.28	2.8	pass
3	7.32	7.93	7.7	pass
4	4.25	4.51	5.8	pass
5	4.19	4.47	6.3	pass
6	7.72	8.47	8.9	pass
7	2.85	2.84	0.4	pass
8	4.26	4.40	3.2	pass
9	4.12	4.25	3.1	pass
10	7.72	7.46	3.5	pass
11	7.31	7.92	7.7	pass
12	4.16	4.38	5.0	pass
13	6.76	7.12	5.1	pass

Appendix 9.6.1 (continued) CLS Cobas c111 Instrument Validation

Triglycerides

	Analyte – Triglycerides, acceptable CV = 20			
	Thistle QA results (mmol/L)	**CLS results (mmol/L)**	**% Deviation**	**Validation outcome**
1	**0.33**	**0.33**	**0.0**	**pass**
2	**1.23**	**1.17**	**5.1**	**pass**
3	**1.07**	**1.09**	**1.8**	**pass**
4	**4.02**	**3.58**	**12.3**	**pass**
5	**2.78**	**2.82**	**1.4**	**pass**
6	**1.11**	**1.05**	**5.7**	**pass**
7	**2.95**	**3.07**	**3.9**	**pass**

Validation acceptance criterion

The % Deviation for the analyte of interest must be less than or equal to the prescribed coefficient of variation (CV).

% Deviation calculation

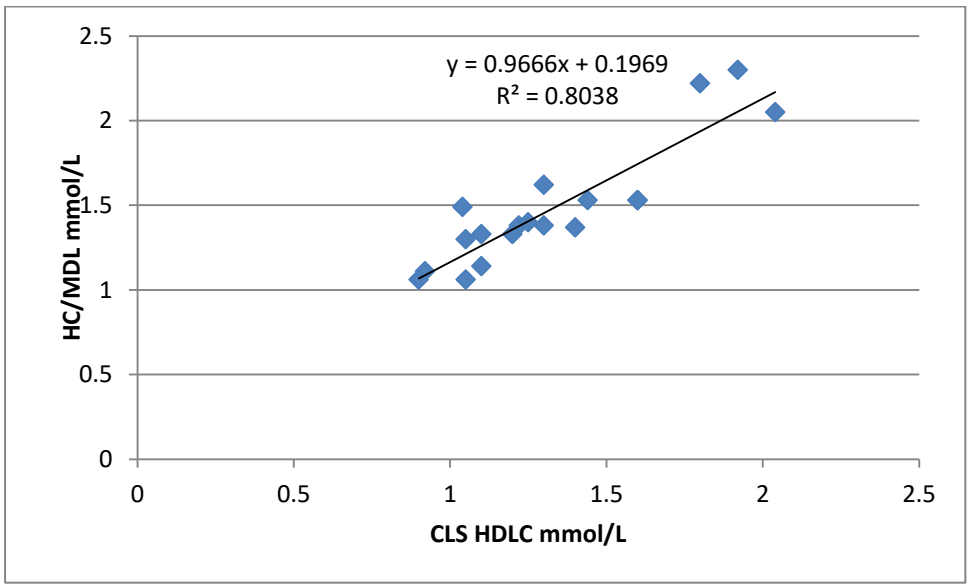
$$\% \text{ Deviation} = \frac{(\text{Thistle EQA instrument mean} - \text{CLS Lab result})}{\text{CLS Lab result}} \times 100$$

Appendix 9.6.2 Inter-laboratory QC

Sequential number	Lab number	EQA enrolled Lab HDLC result (mmol/L)	CLS Lab HDLC result (mmol/L)
1	HC35913	1.44	1.53
2	HC36061	0.92	1.11
3	HC36169	1.22	1.38
4	HC35935	1.05	1.30
5	HC36279	1.25	1.40
6	HC36300	2.04	2.05
7	HC36301	1.05	1.06
8	MDL3862	1.2	1.33
9	MDL3869	1.1	1.14
10	MDL6052	1.3	1.38
11	MDL6043	1.8	2.22
12	MDL6055	1.3	1.62
13	MDL6048	1.1	1.33
14	MDL6053	0.9	1.06
15	MDL6071	1.04	1.49
16	MDL6074	1.92	2.30
17	MDL5973	1.6	1.53
18	MDL5958	1.6	1.53
19	MDL5985	1.4	1.37

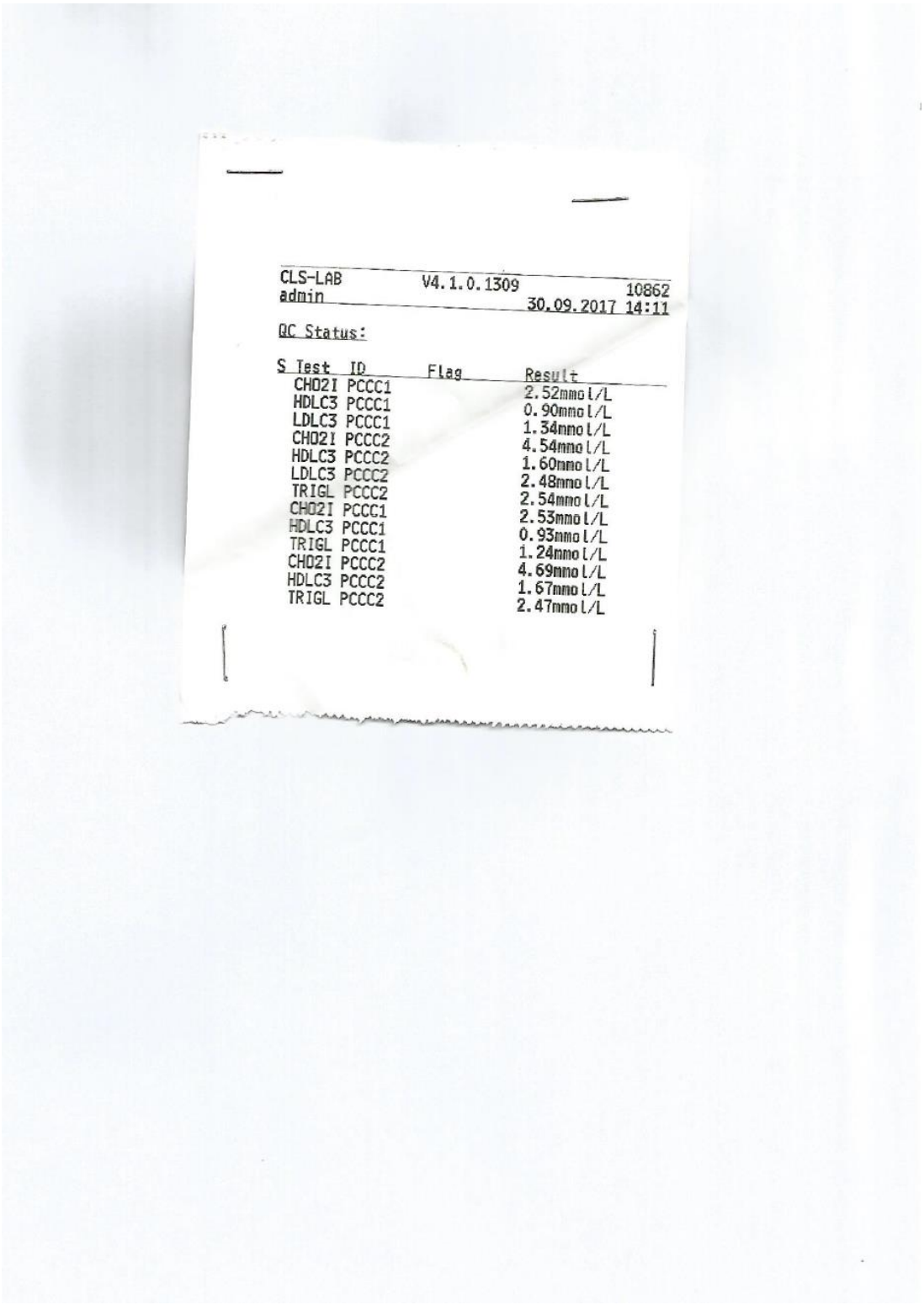
Appendix 9.6.2 (continued) Inter-laboratory (QC)/External Quality Assurance

A linear regression analysis of the HC/MDL HDLC and the CLS HDLC results



Appendix 9.7 Internal QC

Appendix 9.7.1 C111 instrument IQC results print-outs

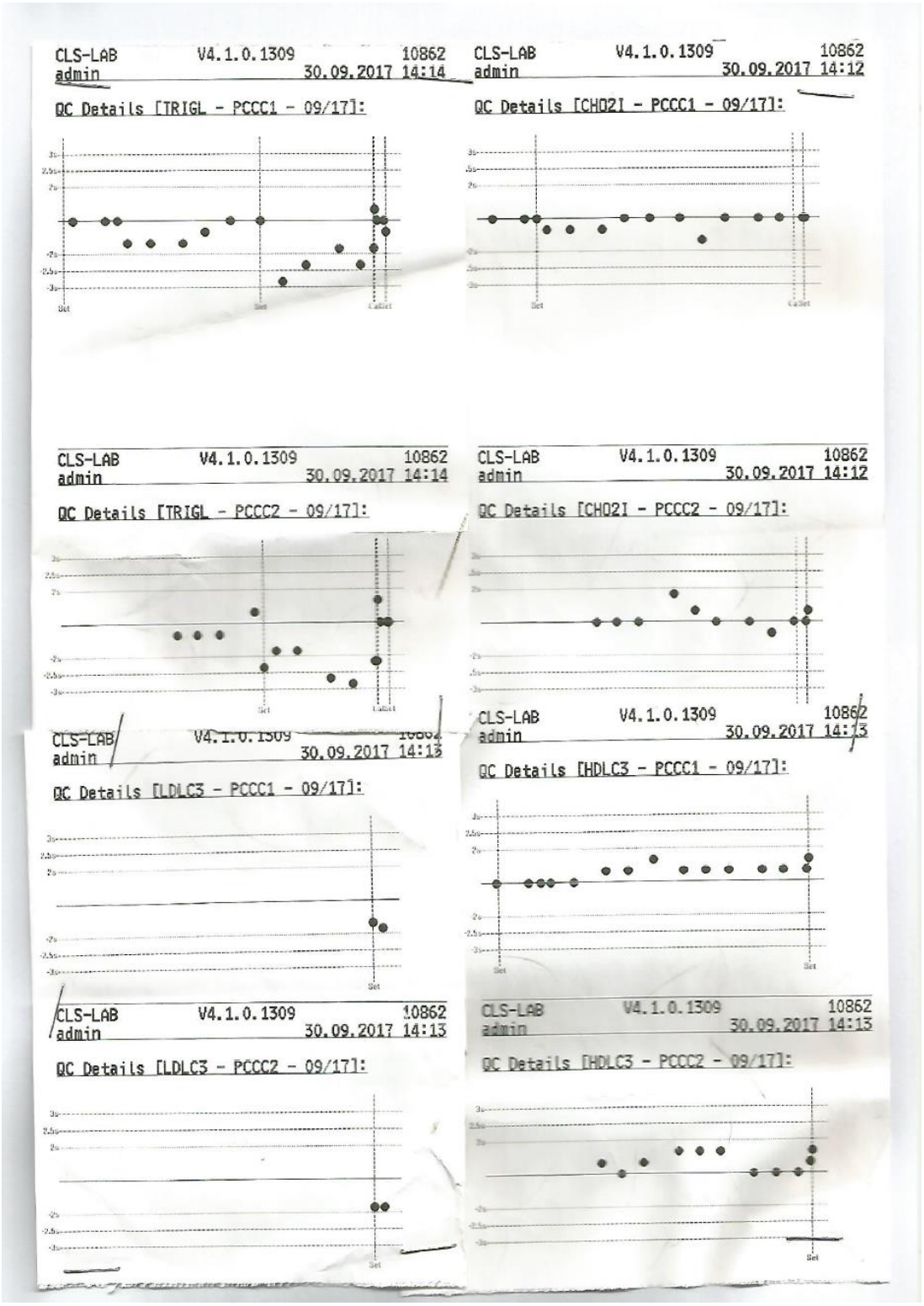


CLS-LAB V4.1.0.1309 10862
admin 30.09.2017 14:11

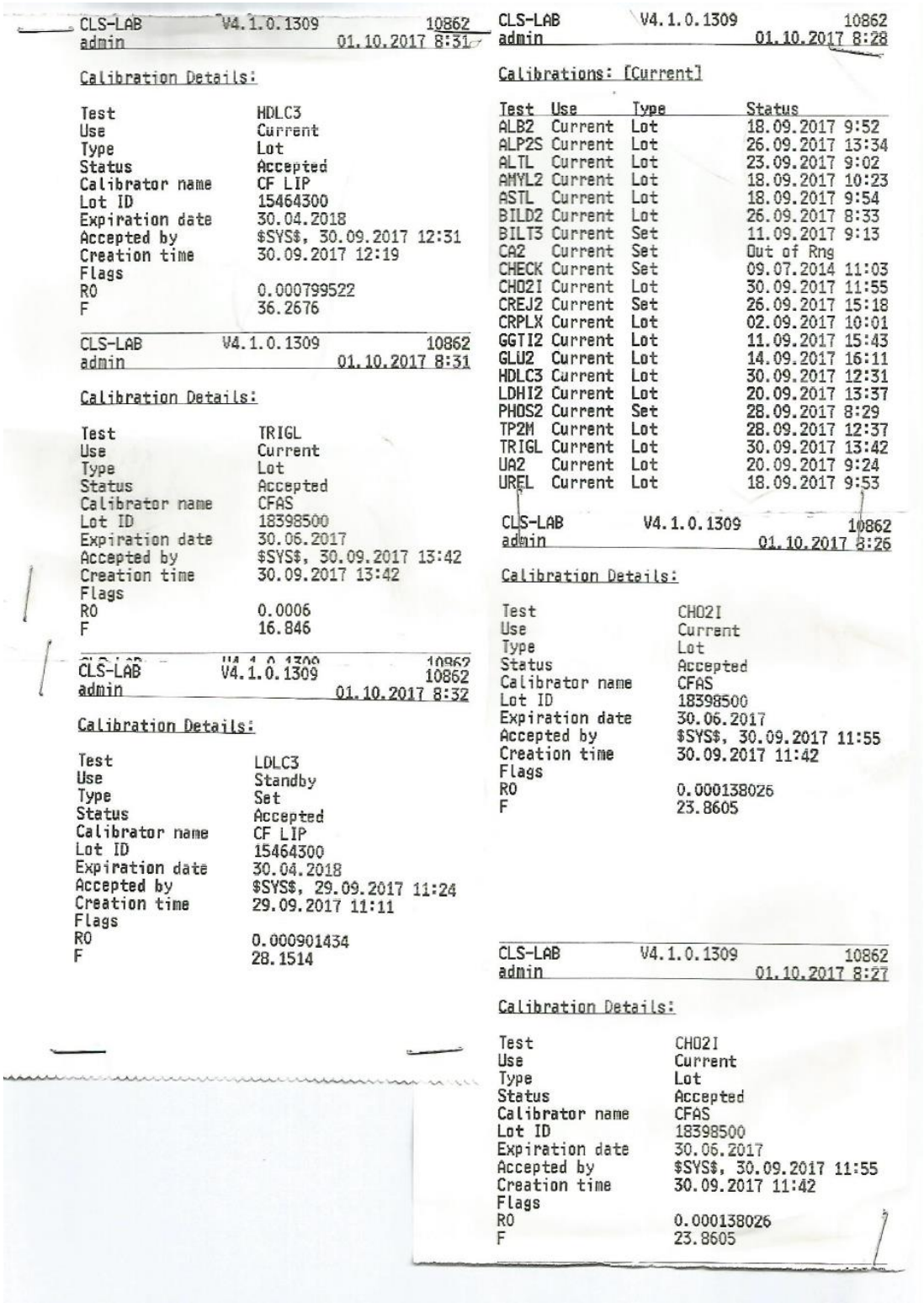
QC Status:

S	Test	ID	Flag	Result
	CHO2I	PCCC1		2.52mmol/L
	HDLC3	PCCC1		0.90mmol/L
	LDLC3	PCCC1		1.34mmol/L
	CHO2I	PCCC2		4.54mmol/L
	HDLC3	PCCC2		1.60mmol/L
	LDLC3	PCCC2		2.48mmol/L
	TRIGL	PCCC2		2.54mmol/L
	CHO2I	PCCC1		2.53mmol/L
	HDLC3	PCCC1		0.93mmol/L
	TRIGL	PCCC1		1.24mmol/L
	CHO2I	PCCC2		4.69mmol/L
	HDLC3	PCCC2		1.67mmol/L
	TRIGL	PCCC2		2.47mmol/L

Appendix 9.7.2 C111 instrument IQC Levy Jennings graphs



Appendix 9.8 C111 instrument calibration reports



CLS-LAB V4.1.0.1309 10862
admin 01.10.2017 8:31

Calibration Details:

Test	HDLC3
Use	Current
Type	Lot
Status	Accepted
Calibrator name	CF LIP
Lot ID	15464300
Expiration date	30.04.2018
Accepted by	SYS, 30.09.2017 12:31
Creation time	30.09.2017 12:19
Flags	
R0	0.000799522
F	36.2676

CLS-LAB V4.1.0.1309 10862
admin 01.10.2017 8:31

Calibration Details:

Test	TRIGL
Use	Current
Type	Lot
Status	Accepted
Calibrator name	CFAS
Lot ID	18398500
Expiration date	30.06.2017
Accepted by	SYS, 30.09.2017 13:42
Creation time	30.09.2017 13:42
Flags	
R0	0.0006
F	16.846

CLS-LAB V4.1.0.1309 10862
admin 01.10.2017 8:32

Calibration Details:

Test	LDLC3
Use	Standby
Type	Set
Status	Accepted
Calibrator name	CF LIP
Lot ID	15464300
Expiration date	30.04.2018
Accepted by	SYS, 29.09.2017 11:24
Creation time	29.09.2017 11:11
Flags	
R0	0.000901434
F	28.1514

CLS-LAB V4.1.0.1309 10862
admin 01.10.2017 8:28

Calibrations: [Current]

Test	Use	Type	Status
ALB2	Current	Lot	18.09.2017 9:52
ALP2S	Current	Lot	26.09.2017 13:34
ALTL	Current	Lot	23.09.2017 9:02
AMYL2	Current	Lot	18.09.2017 10:23
ASTL	Current	Lot	18.09.2017 9:54
BILD2	Current	Lot	26.09.2017 8:33
BILT3	Current	Set	11.09.2017 9:13
CA2	Current	Set	Out of Rng
CHECK	Current	Set	09.07.2014 11:03
CHO2I	Current	Lot	30.09.2017 11:55
CREJ2	Current	Set	26.09.2017 15:18
CRPLX	Current	Lot	02.09.2017 10:01
GGTI2	Current	Lot	11.09.2017 15:43
GLU2	Current	Lot	14.09.2017 16:11
HDLC3	Current	Lot	30.09.2017 12:31
LDHI2	Current	Lot	20.09.2017 13:37
PHOS2	Current	Set	28.09.2017 8:29
TP2M	Current	Lot	28.09.2017 12:37
TRIGL	Current	Lot	30.09.2017 13:42
UA2	Current	Lot	20.09.2017 9:24
UREL	Current	Lot	18.09.2017 9:53

CLS-LAB V4.1.0.1309 10862
admin 01.10.2017 8:26

Calibration Details:

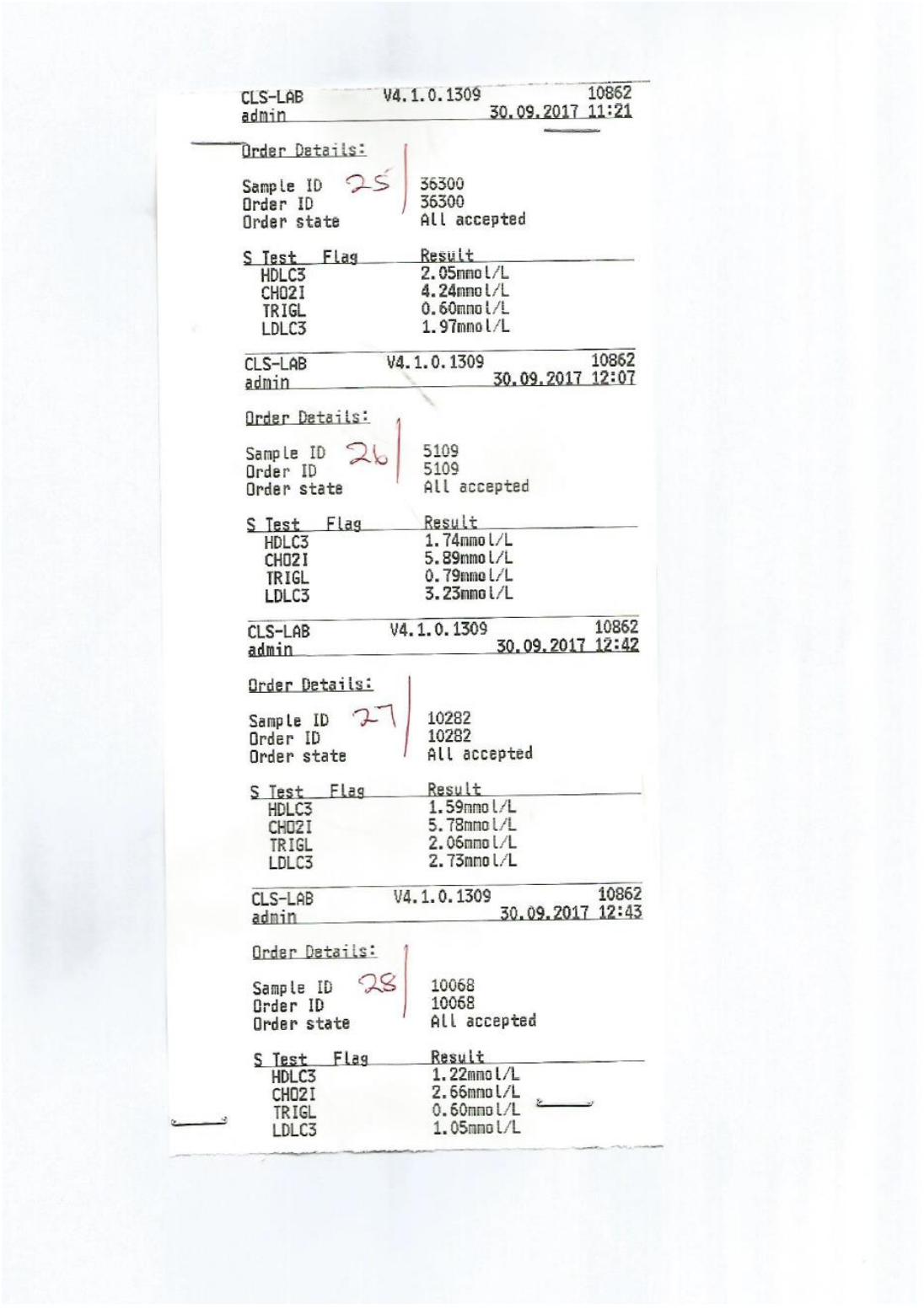
Test	CHO2I
Use	Current
Type	Lot
Status	Accepted
Calibrator name	CFAS
Lot ID	18398500
Expiration date	30.06.2017
Accepted by	SYS, 30.09.2017 11:55
Creation time	30.09.2017 11:42
Flags	
R0	0.000138026
F	23.8605

CLS-LAB V4.1.0.1309 10862
admin 01.10.2017 8:27

Calibration Details:

Test	CHO2I
Use	Current
Type	Lot
Status	Accepted
Calibrator name	CFAS
Lot ID	18398500
Expiration date	30.06.2017
Accepted by	SYS, 30.09.2017 11:55
Creation time	30.09.2017 11:42
Flags	
R0	0.000138026
F	23.8605

Appendix 9.9 c111 instrument patients results print-outs



CLS-LAB V4.1.0.1309 10862
admin 30.09.2017 11:21

Order Details:

25

Sample ID	36300
Order ID	36300
Order state	All accepted

S Test	Flag	Result
HDLC3		2.05mmol/L
CHO2I		4.24mmol/L
TRIGL		0.60mmol/L
LDLC3		1.97mmol/L

CLS-LAB V4.1.0.1309 10862
admin 30.09.2017 12:07

Order Details:

26

Sample ID	5109
Order ID	5109
Order state	All accepted

S Test	Flag	Result
HDLC3		1.74mmol/L
CHO2I		5.89mmol/L
TRIGL		0.79mmol/L
LDLC3		3.23mmol/L

CLS-LAB V4.1.0.1309 10862
admin 30.09.2017 12:42

Order Details:

27

Sample ID	10282
Order ID	10282
Order state	All accepted

S Test	Flag	Result
HDLC3		1.59mmol/L
CHO2I		5.78mmol/L
TRIGL		2.06mmol/L
LDLC3		2.73mmol/L

CLS-LAB V4.1.0.1309 10862
admin 30.09.2017 12:43

Order Details:

28

Sample ID	10068
Order ID	10068
Order state	All accepted

S Test	Flag	Result
HDLC3		1.22mmol/L
CHO2I		2.66mmol/L
TRIGL		0.60mmol/L
LDLC3		1.05mmol/L

Appendix 9.10 Lipid biomarkers results worksheet

Seq. No	Lab ID	Other lipid measurements – mmol/L			Friedewald LDLC (cLDLC)- mmol/L	Roche direct LDLC (dLDLC) – mmol/L	Non-HDLC – mmol/L	sdLDLC – mmol/L	HDLC/cLDLC – mmol/L	HDLC/dLDLC – mmol/L
		TC	TG	HDLC						
1	36301	5.43	0.82	1.06	3.99	3.6	4.37	0.819	0.266	0.294
2	10038	7.56	2.62	1.27	5.08	4.69	6.29	1.589	0.250	0.271
3	5231	5.61	1.61	0.87	4	3.65	4.74	1.046	0.218	0.238
4	10309	5.99	3.58	0.55	3.79	3.17	5.44	1.400	0.145	0.174
5	960	5.2	1.56	1.07	3.41	3.23	4.13	0.947	0.314	0.331
6	10186	8.01	1.34	1.45	5.94	5.65	6.56	1.521	0.244	0.257
7	5191	4.66	0.87	1.42	2.84	2.51	3.24	0.548	0.500	0.566
8	10775	6.43	0.43	2.16	4.07	3.9	4.27	0.829	0.531	0.554
9	10793	5.89	1.33	1.4	3.88	3.69	4.49	1.005	0.361	0.379
10	10276	6.29	1.37	1.08	4.58	4.12	5.21	1.092	0.236	0.262
11	10992	4.08	0.8	0.92	2.79	2.63	3.16	0.588	0.330	0.349
12	85	5.11	0.9	0.91	3.79	3.44	4.2	0.800	0.240	0.265
13	10853	5.8	1.26	1.23	3.99	3.55	4.57	0.913	0.308	0.346
14	5055	3.2	0.49	1.46	1.51	1.58	1.74	0.260	0.967	0.924
15	10908	3.71	0.66	1.19	2.22	2.13	2.52	0.424	0.536	0.559
16	10837	4.71	2.54	0.94	2.6	2.4	3.77	0.980	0.362	0.392
17	5144	4.79	0.85	1.72	2.68	2.43	2.77	0.360	0.642	0.708
18	10005	4.3	1.74	0.84	2.66	2.39	3.46	0.754	0.316	0.351
19	10269	6.67	0.89	1.24	5.02	4.65	5.43	1.121	0.247	0.267
20	5108	6.21	2.29	0.84	4.31	4.03	5.37	1.342	0.195	0.208
21	5247	3.65	0.85	1.38	1.88	1.82	2.3	0.414	0.734	0.758
22	10418	3.19	0.69	1.12	1.75	1.82	2.07	0.376	0.640	0.615
23	389	4.41	0.7	1.88	2.21	2.29	2.53	0.504	0.851	0.821
24	5260	3.33	1.57	0.83	1.78	1.82	2.5	0.602	0.466	0.456
25	36300	4.24	0.6	2.05	1.91	1.97	2.19	0.392	1.073	1.041
26	5109	5.89	0.79	1.74	3.79	3.23	4.15	0.683	0.459	0.539
27	10282	5.78	2.06	1.59	3.24	2.73	4.19	0.896	0.491	0.582
28	10068	2.66	0.6	1.22	1.16	1.05	1.44	0.120	1.052	1.162
29	36279	5.44	1.18	1.4	3.5	2.9	4.04	0.692	0.400	0.483
30	10774	6.14	1.14	1.42	4.19	3.8	4.72	0.959	0.339	0.374
31	35913	5.2	0.56	1.53	3.41	2.97	3.67	0.574	0.449	0.515
32	10656	3.11	0.61	1.57	1.26	1.27	1.54	0.197	1.246	1.236
33	36169	5.68	1.24	1.38	3.73	3.33	4.3	0.855	0.370	0.414
34	5161	5.72	1.08	0.8	4.42	3.88	4.92	0.941	0.181	0.206
35	10295	5.31	1.08	1.29	3.52	2.92	4.02	0.675	0.366	0.442
36	10792	6.4	1.16	1.36	4.51	4.04	5.04	1.011	0.302	0.337
37	36135	4.06	0.61	1.3	2.48	2.23	2.76	0.415	0.524	0.583
38	10340	4.08	1.14	1.06	2.49	2.21	3.02	0.549	0.426	0.480
39	36061	6.13	1.34	1.11	4.4	3.8	5.02	0.980	0.252	0.292

Appendix 9.10 (continued) Lipid biomarkers results worksheet

Seq. No	Lab ID	Other lipid measurements – mmol/L			Friedewald LDLC (cLDLC)- mmol/L	Roche direct LDLC (dLDLC) – mmol/L	Non-HDLC – mmol/L	sdLDLC – mmol/L	HDLC/cLDLC – mmol/L	HDLC/dLDLC – mmol/L
		TC	TG	HDLC						
40	1759	3.77	0.83	1.45	1.94	1.91	2.32	0.420	0.747	0.759
41	12023	7.80	1.53	1.81	5.28	5.05	5.99	1.421	0.342	0.358
42	1988	5.21	0.94	1.73	3.05	2.96	3.48	0.721	0.567	0.584
43	1798	5.90	1.05	1.37	4.05	3.88	4.53	0.985	0.338	0.353
44	11587	4.78	1.77	0.85	3.11	2.92	3.93	0.920	0.273	0.291
45	5491	7.51	0.98	1.60	5.46	5.21	5.91	1.312	0.293	0.307
46	11826	3.10	0.84	1.37	1.34	1.33	1.73	0.273	1.022	1.030
47	11814	5.29	1.19	1.12	3.62	3.59	4.17	0.968	0.309	0.312
48	11635	4.43	0.62	2.05	2.09	2.11	2.38	0.429	0.980	0.972
49	11813	4.49	0.65	1.60	2.59	2.62	2.89	0.573	0.618	0.611
50	11827	4.41	0.93	1.29	2.69	2.68	3.12	0.658	0.450	0.481
51	12414	5.07	0.62	1.27	3.51	3.63	3.80	0.851	0.362	0.350
52	12423	3.70	2.60	0.36	2.14	1.34	3.34	0.624	0.168	0.269
53	12229	6.30	1.14	1.50	4.27	4.30	4.80	1.156	0.351	0.349
54	22110	3.71	0.78	1.16	2.19	2.07	2.55	0.438	0.530	0.560
55	22099	4.95	2.99	0.87	2.70	3.04	4.08	1.353	0.322	0.286
56	22091	4.80	0.63	1.29	3.22	3.03	3.51	0.644	0.400	0.426
57	22088	6.05	1.52	1.14	4.21	4.18	4.91	1.212	0.271	0.273
58	22087	5.97	1.39	1.15	4.18	4.20	4.82	1.191	0.275	0.274
59	22077	5.58	2.87	0.88	3.38	3.39	4.70	1.363	0.260	0.260
60	22034	5.96	4.15	0.91	3.14	3.37	5.05	1.730	0.290	0.270
61	22063	3.22	0.56	1.02	1.94	1.75	2.20	0.284	0.526	0.583
62	22042	4.24	1.04	0.96	2.80	2.40	3.28	0.554	0.343	0.400
63	22041	4.01	1.48	0.79	2.54	2.27	3.22	0.653	0.311	0.348
64	22071	8.54	3.28	1.30	5.73	5.29	7.24	1.914	0.227	0.246
65	22066	4.37	0.83	0.90	3.09	2.87	3.47	0.649	0.291	0.314
66	22064	4.87	1.92	0.97	3.02	2.99	3.90	0.997	0.321	0.324
67	22065	4.90	2.22	1.07	2.81	2.69	3.83	0.984	0.381	0.398
68	22072	4.39	1.38	1.07	2.68	2.55	3.32	0.726	0.399	0.420
69	12494	3.51	2.01	1.04	1.54	1.50	2.47	0.625	0.675	0.693
70	12514	4.47	0.75	1.09	3.03	3.07	3.38	0.724	0.360	0.355
71	12493	5.34	1.81	1.82	2.69	2.59	3.52	0.850	0.677	0.703
72	5833	4.90	1.13	1.774	2.61	2.61	3.13	0.692	0.678	0.678
73	12520	4.69	1.24	1.46	2.66	2.85	3.23	0.814	0.549	0.512
74	12528	5.38	1.30	1.27	3.51	3.36	4.11	0.917	0.362	0.378
75	12524	4.26	1.44	1.78	1.82	2.01	2.48	0.640	0.978	0.886
76	22100	6.02	1.14	0.94	4.55	4.48	5.08	1.189	0.207	0.210
77	22098	5.37	1.47	2.05	2.64	2.75	3.32	0.838	0.777	0.745
78	22078	4.73	2.30	0.97	2.70	2.80	3.76	1.069	0.359	0.346

Appendix 9. 10 (continued) Lipid biomarkers results worksheet

Seq. No	Lab ID	Other lipid measurements – mmol/L			Friedewald LDLC (cLDLC)- mmol/L	Roche direct LDLC (dLDLC) – mmol/L	Non-HDLC – mmol/L	sdLDLC – mmol/L	HDLC/cLDLC – mmol/L	HDLC/dLDLC – mmol/L
		TC	TG	HDLC						
79	22106	7.07	1.02	2.13	4.47	4.76	4.94	1.283	0.477	0.447
80	22105	5.77	1.22	0.93	4.28	4.12	4.84	1.096	0.217	0.226
81	12561	4.77	0.88	1.12	3.24	2.83	3.65	0.627	0.346	0.396
82	12552	5.45	1.77	1.27	3.36	2.81	4.18	0.837	0.378	0.452
83	22142	5.56	3.01	1.17	3.00	2.64	4.39	1.147	0.390	0.443
84	22140	6.46	1.00	1.84	4.16	3.29	4.62	0.711	0.442	0.559
85	22143	5.05	1.33	1.09	3.35	2.74	3.96	0.688	0.325	0.398
86	22144	5.89	0.90	1.24	4.24	3.45	4.65	0.737	0.292	0.359
87	22231	4.63	6.81	0.45	1.04	1.31	4.18	1.891	0.433	0.344
88	22191	5.60	0.74	1.37	3.89	3.32	4.23	0.694	0.352	0.413
89	22152	3.44	2.03	1.19	1.31	1.26	2.25	0.565	0.908	0.944
90	22155	6.12	3.52	0.79	3.71	3.42	5.33	1.499	0.213	0.231
91	22157	6.52	0.93	3.14	2.95	2.53	3.38	0.557	1.064	1.241
92	22207	8.76	2.97	0.61	6.78	5.68	8.15	1.840	0.090	0.107
93	22208	6.59	1.75	1.48	4.30	3.71	5.11	1.066	0.344	0.399
94	22230	4.41	1.28	1.62	2.20	1.99	2.79	0.535	0.736	0.814
95	22206	8.37	1.22	1.77	6.04	5.08	6.60	1.234	0.293	0.348
96	22189	5.60	1.09	1.46	3.64	3.10	4.14	0.731	0.401	0.471
97	22237	6.18	3.04	0.86	3.92	3.08	5.32	1.199	0.219	0.279
98	22218	5.15	2.87	1.37	2.46	2.37	3.78	1.075	0.557	0.578
99	22188	4.41	0.83	1.35	2.68	2.34	3.06	0.489	0.504	0.577
100	22167	5.20	0.77	1.60	3.25	2.78	3.60	0.570	0.492	0.576
101	22164	6.13	2.00	1.42	3.79	3.27	4.71	1.022	0.375	0.434
102	22168	6.23	3.85	0.69	3.77	3.30	5.54	1.526	0.183	0.209
103	22171	3.55	0.97	0.75	2.35	1.95	2.80	0.416	0.319	0.385
104	22187	3.27	0.60	0.90	2.09	1.73	2.37	0.265	0.431	0.520
105	22186	3.29	0.46	1.03	2.05	1.72	2.26	0.227	0.502	0.599
106	22184	6.21	1.91	1.01	4.32	3.56	5.20	1.041	0.234	0.284
107	22267	5.09	2.46	0.81	3.15	2.90	4.28	1.084	0.257	0.279
108	22334	4.24	0.45	1.55	2.48	2.16	2.69	0.346	0.625	0.718
109	22352	4.54	1.47	0.80	3.06	2.58	3.74	0.705	0.261	0.310
110	22323	4.85	1.40	1.21	2.99	2.60	3.64	0.706	0.405	0.465
111	22322	4.54	1.49	0.79	3.06	2.53	3.75	0.690	0.258	0.312
112	22351	5.78	6.76	0.60	2.06	2.88	5.18	2.382	0.291	0.208
113	22360	4.51	0.78	1.32	2.83	2.53	3.19	0.534	0.466	0.522
114	22335	7.20	1.45	0.80	5.73	4.95	6.40	1.290	0.140	0.162
115	22307	3.24	1.17	0.76	1.94	1.60	2.48	0.383	0.392	0.475
116	22308	5.00	2.96	0.68	2.96	2.74	4.32	1.178	0.230	0.248
117	22320	5.64	0.80	1.85	3.42	2.91	3.79	0.611	0.541	0.636

Appendix 9.10 (continued) Lipid biomarkers results worksheet

Seq. No	Lab ID	Other lipid measurements – mmol/L			Friedewald LDLC (cLDLC)- mmol/L	Roche direct LDLC (dLDLC) – mmol/L	Non-HDLC – mmol/L	sdLDLC – mmol/L	HDLC/cLDLC – mmol/L	HDLC/dLDLC – mmol/L
		TC	TG	HDLC						
118	22318	5.33	0.92	1.46	3.45	3.06	3.87	0.697	0.423	0.477
119	22330	6.26	2.35	1.11	4.07	3.66	5.15	1.235	0.273	0.303
120	22327	4.25	1.48	0.73	2.84	2.34	3.52	0.638	0.257	0.312
121	22328	6.08	3.48	1.00	3.48	3.52	5.08	1.563	0.287	0.284
122	12577	6.33	2.44	1.08	4.13	3.51	5.25	1.186	0.262	0.308
123	12589	4.79	0.90	1.17	3.21	3.33	3.62	0.840	0.364	0.351

Appendix 9.11 A comparison of Friedewald method calculated LDLC (cLDLC) and Roche Diagnostics direct method LDLC (dLDLC) values and their risk categories

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		Roche Diagnostics LDLC (dLDLC)			Change in risk category/status
		value	risk category	value	risk category		
1	36301	3.99	BH	3.6	BH	↓	
2	10038	5.08	VH	4.69	H	↓	↓, 1 category
3	5231	4	BH	3.65	BH	↓	
4	10309	3.79	BH	3.17	N/AO	↓	↓, 1 category
5	960	3.41	BH	3.23	N/AO	↓	↓, 1 category
6	10186	5.94	VH	5.65	VH	↓	
7	5191	2.84	N/AO	2.51	O	↓	↓, 1 category
8	10775	4.07	BH	3.9	BH	↓	
9	10793	3.88	BH	3.69	BH	↓	
10	10276	4.58	H	4.12	BH	↓	↓, 1 category
11	10992	2.79	N/AO	2.63	N/AO	↓	
12	85	3.79	BH	3.44	BH	↓	
13	10853	3.99	BH	3.55	BH	↓	
14	5055	1.51	O	1.58	O	↑	
15	10908	2.22	O	2.13	O	↓	
16	10837	2.6	N/AO	2.4	O	↓	↓, 1 category
17	5144	2.68	N/AO	2.43	O	↓	↓, 1 category
18	10005	2.66	N/AO	2.39	O	↓	↓, 1 category
19	10269	5.02	VH	4.65	H	↓	↓, 1 category
20	5108	4.31	H	4.03	BH	↓	↓, 1 category
21	5247	1.88	O	1.82	O	↓	
22	10418	1.75	O	1.82	O	↑	
23	389	2.21	O	2.29	O	↑	
24	5260	1.78	O	1.82	O	↑	
25	36300	1.91	O	1.97	O	↑	
26	5109	3.79	BH	3.23	N/AO	↓	↓, 1 category
27	10282	3.24	N/AO	2.73	N/AO	↓	
28	10068	1.16	O	1.05	O	↓	
29	36279	3.5	BH	2.9	N/AO	↓	↓, 1 category
30	10774	4.19	H	3.8	BH	↓	↓, 1 category
31	35913	3.41	BH	2.97	N/AO	↓	↓, 1 category
32	10656	1.26	O	1.27	O	↑	
33	36169	3.73	BH	3.33	N/AO	↓	↓, 1 category
34	5161	4.42	H	3.88	BH	↓	↓, 1 category
35	10295	3.52	BH	2.92	N/AO	↓	↓, 1 category
36	10792	4.51	H	4.04	H	↓	
37	36135	2.48	O	2.23	O	↓	
38	10340	2.49	O	2.21	O	↓	
39	36061	4.4	H	3.8	BH	↓	↓, 1 category

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high, ↑- increase, ↓ - decrease.

Appendix 9.11 (continued) A comparison of Friedewald method calculated LDLC (cLDLC) and Roche Diagnostics direct method LDLC (dLDLC) values and their risk categories

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		Roche Diagnostics LDLC (dLDLC)			Change in risk category/status
		value	risk category	value	risk category		
40	1759	1.94	O	1.91	O	↓	
41	12023	5.28	VH	5.05	VH	↓	
42	1988	3.05	N/AO	2.96	N/AO	↓	
43	1798	4.05	BH	3.88	BH	↓	
44	11587	3.11	N/AO	2.92	N/AO	↓	
45	5491	5.46	VH	5.21	VH	↓	
46	11826	1.34	O	1.33	O	↓	
47	11814	3.62	BH	3.59	BH	↓	
48	11635	2.09	O	2.11	O	↑	
49	11813	2.59	N/AO	2.62	N/AO	↑	
50	11827	2.69	N/AO	2.68	N/AO	↓	
51	12414	3.51	BH	3.63	BH	↑	
52	12423	2.14	O	1.34	O	↓	
53	12229	4.27	H	4.30	H	↑	
54	22110	2.19	O	2.07	O	↓	
55	22099	2.70	N/AO	3.04	N/AO	↑	
56	22091	3.22	N/AO	3.03	N/AO	↓	
57	22088	4.21	H	4.18	H	↓	
58	22087	4.18	H	4.20	H	↑	
59	22077	3.38	BH	3.39	BH	↑	
60	22034	3.14	N/AO	3.37	BH	↑	↑, 1 category
61	22063	1.94	O	1.75	O	↓	
62	22042	2.80	N/AO	2.40	O	↓	↓, 1 category
63	22041	2.54	O	2.27	O	↓	
64	22071	5.73	VH	5.29	VH	↓	
65	22066	3.09	N/AO	2.87	N/AO	↓	
66	22064	3.02	N/AO	2.99	N/AO	↓	
67	22065	2.81	N/AO	2.69	N/AO	↓	
68	22072	2.68	N/AO	2.55	O	↓	↓, 1 category
69	12494	1.54	O	1.50	O	↓	
70	12514	3.03	N/AO	3.07	N/AO	↑	
71	12493	2.69	N/AO	2.59	N/AO	↓	
72	5833	2.61	N//AO	2.61	N/AO	---	
73	12520	2.66	N/AO	2.85	N/AO	↑	
74	12528	3.51	BH	3.36	BH	↓	
75	12524	1.82	O	2.01	O	↑	
76	22100	4.55	H	4.48	H	↓	
77	22098	2.64	N/AO	2.75	N/AO	↑	
78	22078	2.70	N/AO	2.80	N/AO	↑	

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high, ↑- increase, ↓ - decrease.

Appendix 9.11 (continued) A comparison of Friedewald method calculated LDLC (cLDLC) and Roche Diagnostics direct method LDLC (dLDLC) values and their risk categories

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		Roche Diagnostics LDLC (dLDLC)			Change in risk category/status
		value	risk category	value	risk category		
79	22106	4.47	H	4.76	H	↑	
80	22105	4.28	H	4.12	BH	↓	↓, 1 category
81	12561	3.24	N/AO	2.83	N/AO	↓	
82	12552	3.36	BH	2.81	N/AO	↓	↓, 1 category
83	22142	3.00	N/AO	2.64	N/AO	↓	
84	22140	4.16	H	3.29	N/AO	↓	↓, 2 categories
85	22143	3.35	BH	2.74	N/AO	↓	↓, 1 category
86	22144	4.24	H	3.45	BH	↓	↓, 1 category
87	22231	1.04	O	1.31	O	↑	Excluded sample
88	22191	3.89	BH	3.32	N/AO	↓	↓, 1 category
89	22152	1.31	O	1.26	O	↓	
90	22155	3.71	BH	3.42	BH	↓	
91	22157	2.95	N/AO	2.53	O	↓	↓, 1 category
92	22207	6.78	VH	5.68	VH	↓	
93	22208	4.30	H	3.71	BH	↓	↓, 1 category
94	22230	2.20	O	1.99	O	↓	
95	22206	6.04	VH	5.08	VH	↓	
96	22189	3.64	BH	3.10	N/AO	↓	↓, 1 category
97	22237	3.92	BH	3.08	N/AO	↓	↓, 1 category
98	22218	2.46	O	2.37	O	↓	
99	22188	2.68	N/AO	2.34	O	↓	↓, 1 category
100	22167	3.25	N/AO	2.78	N/AO	↓	
101	22164	3.79	BH	3.27	N/AO	↓	↓, 1 category
102	22168	3.77	BH	3.30	N/AO	↓	↓, 1 category
103	22171	2.35	O	1.95	O	↓	
104	22187	2.09	O	1.73	O	↓	
105	22186	2.05	O	1.72	O	↓	
106	22184	4.32	H	3.56	BH	↓	↓, 1 category
107	22267	3.15	N/AO	2.90	N/AO	↓	
108	22334	2.48	O	2.16	O	↓	
109	22352	3.06	N/AO	2.58	O	↓	↓, 1 category
110	22323	2.99	N/AO	2.60	N/AO	↓	
111	22322	3.06	N/AO	2.53	O	↓	↓, 1 category
112	22351	2.06		2.88			Excluded sample
113	22360	2.83	N/AO	2.53	O	↓	↓, 1 category
114	22335	5.73	VH	4.95	VH	↓	
115	22307	1.94	O	1.60	O	↓	
116	22308	2.96	N/AO	2.74	N/AO	↓	
117	22320	3.42	BH	2.91	N/AO	↓	↓, 1 category

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high, ↑- increase, ↓ - decrease.

Appendix 9.11 (continued) A comparison of Friedewald method calculated LDLC (cLDLC) and Roche Diagnostics direct method LDLC (dLDLC) values and their risk categories

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		Roche Diagnostics LDLC (dLDLC)			Change in risk category/status
		value	risk category	value	risk category		
118	22318	3.45	BH	3.06	N/AO	↓	↓, 1 category
119	22330	4.07	BH	3.66	BH	↓	
120	22327	2.84	N/AO	2.34	O	↓	↓, 1 category
121	22328	3.48	BH	3.52	BH	↑	
122	12577	4.13	BH	3.51	BH	↓	
123	12589	3.21	N/AO	3.33	N/AO	↑	

Appendix 9.12 A comparison of CV risk establishment using the Friedewald calculated LDLC (cLDLC) and non-HDLC calculation

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		non-HDLC		Change in risk category/status
		value	risk category	value	risk category	
1	36301	3.99	BH	4.37	BH	
2	10038	5.08	VH	6.29	VH	
3	5231	4	BH	4.74	BH	
4	10309	3.79	BH	5.44	H	
5	960	3.41	BH	4.13	BH	
6	10186	5.94	VH	6.56	VH	
7	5191	2.84	N/AO	3.24	O	↓, 1 category
8	10775	4.07	BH	4.27	BH	
9	10793	3.88	BH	4.49	BH	
10	10276	4.58	H	5.21	H	
11	10992	2.79	N/AO	3.16	O	↓, 1 category
12	85	3.79	BH	4.2	BH	
13	10853	3.99	BH	4.57	BH	
14	5055	1.51	O	1.74	O	
15	10908	2.22	O	2.52	O	
16	10837	2.6	N/AO	3.77	N/AO	
17	5144	2.68	N/AO	2.77	O	↓, 1 category
18	10005	2.66	N/AO	3.46	N/AO	
19	10269	5.02	VH	5.43	H	↓, 1 category
20	5108	4.31	H	5.37	H	
21	5247	1.88	O	2.3	O	
22	10418	1.75	O	2.07	O	
23	389	2.21	O	2.53	O	
24	5260	1.78	O	2.5	O	
25	36300	1.91	O	2.19	O	
26	5109	3.79	BH	4.15	BH	
27	10282	3.24	N/AO	4.19	BH	↑, 1 category
28	10068	1.16	O	1.44	O	
29	36279	3.5	BH	4.04	N/AO	↓,1 category
30	10774	4.19	H	4.72	BH	↓, 1 category
31	35913	3.41	BH	3.67	N/AO	↓, 1 category
32	10656	1.26	O	1.54	O	
33	36169	3.73	BH	4.3	BH	
34	5161	4.42	H	4.92	H	
35	10295	3.52	BH	4.02	N/AO	↓, 1 category
36	10792	4.51	H	5.04	H	
37	36135	2.48	O	2.76	O	
38	10340	2.49	O	3.02	O	
39	36061	4.4	H	5.02	H	

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high,

↑- increase, ↓ - decrease.

Appendix 9.12 (continued) A comparison of CV risk establishment using the Friedewald calculated LDLC (cLDLC) and non-HDLC calculation

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		non-HDLC		Change in risk category/status
		value	risk category	value	risk category	
40	1759	1.94	O	2.32	O	
41	12023	5.28	VH	5.99	VH	
42	1988	3.05	N/AO	3.48	N/AO	
43	1798	4.05	BH	4.53	BH	
44	11587	3.11	N/AO	3.93	N/AO	
45	5491	5.46	VH	5.91	VH	
46	11826	1.34	O	1.73	O	
47	11814	3.62	BH	4.17	BH	
48	11635	2.09	O	2.38	O	
49	11813	2.59	N/AO	2.89	O	↓,1 category
50	11827	2.69	N/AO	3.12	O	↓,1 category
51	12414	3.51	BH	3.80	N/AO	↓,1 category
52	12423	2.14	O	3.34	O	
53	12229	4.27	H	4.80	BH	↓,1 category
54	22110	2.19	O	2.55	O	
55	22099	2.70	N/AO	4.08	N/AO	
56	22091	3.22	N/AO	3.51	N/AO	
57	22088	4.21	H	4.91	H	
58	22087	4.18	H	4.82	BH	↓,1 category
59	22077	3.38	BH	4.70	BH	
60	22034	3.14	N/AO	5.05	H	↑, 2 categories
61	22063	1.94	O	2.20	O	
62	22042	2.80	N/AO	3.28	O	↓,1 category
63	22041	2.54	O	3.22	O	
64	22071	5.73	VH	7.24	VH	
65	22066	3.09	N/AO	3.47	N/AO	
66	22064	3.02	N/AO	3.90	N/AO	
67	22065	2.81	N/AO	3.83	N/AO	
68	22072	2.68	N/AO	3.32	O	↓,1 category
69	12494	1.54	O	2.47	O	
70	12514	3.03	N/AO	3.38	N/AO	
71	12493	2.69	N/AO	3.52	N/AO	
72	5833	2.61	N//AO	3.13	O	↓,1 category
73	12520	2.66	N/AO	3.23	O	↓,1 category
74	12528	3.51	BH	4.11	N/AO	↓,1 category
75	12524	1.82	O	2.48	O	
76	22100	4.55	H	5.08	H	
77	22098	2.64	N/AO	3.32	O	↓,1 category
78	22078	2.70	N/AO	3.76	N/AO	

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high,

↑- increase, ↓ - decrease.

Appendix 9.12 (continued) A comparison of CV risk establishment using the Friedewald calculated LDLC (cLDLC) and non-HDLC calculation

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		non-HDLC		Change in risk category/status
		value	risk category	value	risk category	
79	22106	4.47	H	4.94	H	
80	22105	4.28	H	4.84	BH	↓,1 category
81	12561	3.24	N/AO	3.65	N/AO	
82	12552	3.36	BH	4.18	BH	
83	22142	3.00	N/AO	4.39	BH	↑, 1 category
84	22140	4.16	H	4.62	BH	↓, 1 category
85	22143	3.35	BH	3.96	N/AO	↓, 1 category
86	22144	4.24	H	4.65	BH	↓, 1 category
87	22231	1.04		4.18		Excluded sample
88	22191	3.89	BH	4.23	BH	
89	22152	1.31	O	2.25	O	
90	22155	3.71	BH	5.33	H	↑, 1 category
91	22157	2.95	N/AO	3.38	N/AO	
92	22207	6.78	VH	8.15	VH	
93	22208	4.30	H	5.11	H	
94	22230	2.20	O	2.79	O	
95	22206	6.04	VH	6.60	VH	
96	22189	3.64	BH	4.14	BH	
97	22237	3.92	BH	5.32	H	↑, 1 category
98	22218	2.46	O	3.78	N/AO	↑, 1 category
99	22188	2.68	N/AO	3.06	O	↓, 1 category
100	22167	3.25	N/AO	3.60	N/AO	
101	22164	3.79	BH	4.71	BH	
102	22168	3.77	BH	5.54	H	↑, 1 category
103	22171	2.35	O	2.80	O	
104	22187	2.09	O	2.37	O	
105	22186	2.05	O	2.26	O	
106	22184	4.32	H	5.20	H	
107	22267	3.15	N/AO	4.28	BH	↑, 1 category
108	22334	2.48	O	2.69	O	
109	22352	3.06	N/AO	3.74	N/AO	
110	22323	2.99	N/AO	3.64	N/AO	
111	22322	3.06	N/AO	3.75	N/AO	
112	22351	2.06		5.18		Excluded sample
113	22360	2.83	N/AO	3.19	O	↓, 1 category
114	22335	5.73	VH	6.40	VH	
115	22307	1.94	O	2.48	O	
116	22308	2.96	N/AO	4.32	BH	↑, 1 category
117	22320	3.42	BH	3.79	N/AO	↓, 1 category

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high,

↑- increase, ↓ - decrease.

Appendix 9.12 (continued) A comparison of CV risk establishment using the Friedewald calculated LDLC (cLDLC) and non-HDLC calculation

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		non-HDLC		Change in risk category/status
		value	risk category	value	risk category	
118	22318	3.45	BH	3.87	N/AO	↓, 1 category
119	22330	4.07	BH	5.15	H	↑, 1 category
120	22327	2.84	N/AO	3.52	N/AO	
121	22328	3.48	BH	5.08	H	↑, 1 category
122	12577	4.13	BH	5.25	H	↑, 1 category
123	12589	3.21	N/AO	3.62	N/AO	

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high,

↑- increase, ↓ - decrease.

Appendix 9.13 A comparison of CV risk establishment using the Roche Diagnostics direct LDLC method (dLDLC) and the non – HDLC calculation

Sequential number	Lab ID	Roche Diagnostics LDLC (dLDLC)		non-HDLC		Change in risk category/status
		value	risk category	value	risk category	
1	36301	3.6	BH	4.37	BH	
2	10038	4.69	H	6.29	VH	↑, 1 category
3	5231	3.65	BH	4.74	BH	
4	10309	3.17	N/AO	5.44	H	↑, 2 categories
5	960	3.23	N/AO	4.13	BH	↑, 1 category
6	10186	5.65	VH	6.56	VH	
7	5191	2.51	O	3.24	O	
8	10775	3.9	BH	4.27	BH	
9	10793	3.69	BH	4.49	BH	
10	10276	4.12	BH	5.21	H	
11	10992	2.63	N/AO	3.16	O	↓, 1 category
12	85	3.44	BH	4.2	BH	
13	10853	3.55	BH	4.57	BH	
14	5055	1.58	O	1.74	O	
15	10908	2.13	O	2.52	O	
16	10837	2.4	O	3.77	N/AO	↑, 1 category
17	5144	2.43	O	2.77	O	
18	10005	2.39	O	3.46	N/AO	↑, 1 category
19	10269	4.65	H	5.43	H	
20	5108	4.03	BH	5.37	H	↑, 1 category
21	5247	1.82	O	2.3	O	
22	10418	1.82	O	2.07	O	
23	389	2.29	O	2.53	O	
24	5260	1.82	O	2.5	O	
25	36300	1.97	O	2.19	O	
26	5109	3.23	N/AO	4.15	BH	↑, 1 category
27	10282	2.73	N/AO	4.19	BH	↑, 1 category
28	10068	1.05	O	1.44	O	
29	36279	2.9	N/AO	4.04	N/AO	
30	10774	3.8	BH	4.72	BH	
31	35913	2.97	N/AO	3.67	N/AO	
32	10656	1.27	O	1.54	O	
33	36169	3.33	N/AO	4.3	BH	↑, 1 category
34	5161	3.88	BH	4.92	H	↑, 1 category
35	10295	2.92	N/AO	4.02	N/AO	
36	10792	4.04	H	5.04	H	
37	36135	2.23	O	2.76	O	
38	10340	2.21	O	3.02	O	
39	36061	3.8	BH	5.02	H	↑, 1 category

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high,

↑- increase, ↓ - decrease.

Appendix 9.13 (continued) A comparison of CV risk establishment using the Roche Diagnostics direct LDLC method (dLDLC) and the non – HDLC calculation

Sequential number	Lab ID	Roche Diagnostics LDLC (dLDLC)		non-HDLC		Change in risk category/status
		value	risk category	value	risk category	
40	1759	1.91	O	2.32	O	
41	12023	5.05	VH	5.99	VH	
42	1988	2.96	N/AO	3.48	N/AO	
43	1798	3.88	BH	4.53	BH	
44	11587	2.92	N/AO	3.93	N/AO	
45	5491	5.21	VH	5.91	VH	
46	11826	1.33	O	1.73	O	
47	11814	3.59	BH	4.17	BH	
48	11635	2.11	O	2.38	O	
49	11813	2.62	N/AO	2.89	O	↓, 1 category
50	11827	2.68	N/AO	3.12	O	↓, 1 category
51	12414	3.63	BH	3.80	N/AO	↓, 1 category
52	12423	1.34	O	3.34	O	
53	12229	4.30	H	4.80	BH	↓, 1 category
54	22110	2.07	O	2.55	O	
55	22099	3.04	N/AO	4.08	N/AO	
56	22091	3.03	N/AO	3.51	N/AO	
57	22088	4.18	H	4.91	H	
58	22087	4.20	H	4.82	BH	↓, 1 category
59	22077	3.39	BH	4.70	BH	
60	22034	3.37	BH	5.05	H	↑, 1 category
61	22063	1.75	O	2.20	O	
62	22042	2.40	O	3.28	O	
63	22041	2.27	O	3.22	O	
64	22071	5.29	VH	7.24	VH	
65	22066	2.87	N/AO	3.47	N/AO	
66	22064	2.99	N/AO	3.90	N/AO	
67	22065	2.69	N/AO	3.83	N/AO	
68	22072	2.55	O	3.32	O	
69	12494	1.50	O	2.47	O	
70	12514	3.07	N/AO	3.38	N/AO	
71	12493	2.59	N/AO	3.52	N/AO	
72	5833	2.61	N/AO	3.13	O	↓, 1 category
73	12520	2.85	N/AO	3.23	O	↓, 1 category
74	12528	3.36	BH	4.11	N/AO	↓, 1 category
75	12524	2.01	O	2.48	O	
76	22100	4.48	H	5.08	H	
77	22098	2.75	N/AO	3.32	O	↓, 1 category
78	22078	2.80	N/AO	3.76	N/AO	

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high,

↑- increase, ↓ - decrease.

Appendix 9.13 (continued) A comparison of CV risk establishment using the Roche Diagnostics direct LDLC method (dLDLC) and the non – HDLC calculation

Sequential number	Lab ID	Roche Diagnostics LDLC (dLDLC)		non-HDLC		Change in risk category/status
		value	risk category	value	risk category	
79	22106	4.76	H	4.94	H	
80	22105	4.12	BH	4.84	BH	
81	12561	2.83	N/AO	3.65	N/AO	
82	12552	2.81	N/AO	4.18	BH	↑, 1 category
83	22142	2.64	N/AO	4.39	BH	↑, 1 category
84	22140	3.29	N/AO	4.62	BH	↑, 1 category
85	22143	2.74	N/AO	3.96	N/AO	
86	22144	3.45	BH	4.65	BH	
87	22231	1.31		4.18		Excluded sample
88	22191	3.32	N/AO	4.23	BH	↑, 1 category
89	22152	1.26	O	2.25	O	
90	22155	3.42	BH	5.33	H	↑, 1 category
91	22157	2.53	O	3.38	N/AO	↑, 1 category
92	22207	5.68	VH	8.15	VH	
93	22208	3.71	BH	5.11	H	↑, 1 category
94	22230	1.99	O	2.79	O	
95	22206	5.08	VH	6.60	VH	
96	22189	3.10	N/AO	4.14	BH	↑, 1 category
97	22237	3.08	N/AO	5.32	H	↑, 2 categories
98	22218	2.37	O	3.78	N/AO	↑, 1 category
99	22188	2.34	O	3.06	O	
100	22167	2.78	N/AO	3.60	N/AO	
101	22164	3.27	N/AO	4.71	BH	↑, 1 category
102	22168	3.30	N/AO	5.54	H	↑, 2 categories
103	22171	1.95	O	2.80	O	
104	22187	1.73	O	2.37	O	
105	22186	1.72	O	2.26	O	
106	22184	3.56	BH	5.20	H	↑, 1 category
107	22267	2.90	N/AO	4.28	BH	↑, 1 category
108	22334	2.16	O	2.69	O	
109	22352	2.58	O	3.74	N/AO	↑, 1 category
110	22323	2.60	N/AO	3.64	N/AO	
111	22322	2.53	O	3.75	N/AO	↑, 1 category
112	22351	2.88		5.18		Excluded sample
113	22360	2.53	O	3.19	O	
114	22335	4.95	VH	6.40	VH	
115	22307	1.60	O	2.48	O	
116	22308	2.74	N/AO	4.32	BH	↑, 1 category
117	22320	2.91	N/AO	3.79	N/AO	

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high,

↑- increase, ↓ - decrease.

Appendix 9.13 (continued) A comparison of CV risk establishment using the Roche Diagnostics direct LDLC method (dLDLC) and the non – HDLC calculation

Sequential number	Lab ID	Roche Diagnostics LDLC (dLDLC)		non-HDLC		Change in risk category/status
		value	risk category	value	risk category	
118	22318	3.06	N/AO	3.87	N/AO	
119	22330	3.66	BH	5.15	H	↑, 1 category
120	22327	2.34	O	3.52	N/AO	↑, 1 category
121	22328	3.52	BH	5.08	H	↑, 1 category
122	12577	3.51	BH	5.25	H	↑, 1 category
123	12589	3.33	N/AO	3.62	N/AO	

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high,

↑- increase, ↓ - decrease.

Appendix 9.14 A comparison of CV risk estimation using the non-HDLCA and the non – HDLC

Sequential number	Lab ID	Non-HDLCA)		non-HDLC		Change in risk category/status
		value	risk category	value	risk category	
1	36301	3.59	BH	4.37	BH	
2	10038	5.51	VH	6.29	VH	
3	5231	3.96	BH	4.74	BH	
4	10309	4.66	H	5.44	H	
5	960	3.35	BH	4.13	BH	
6	10186	5.78	VH	6.56	VH	
7	5191	2.46	O	3.24	O	
8	10775	3.49	BH	4.27	BH	
9	10793	3.71	BH	4.49	BH	
10	10276	4.43	H	5.21	H	
11	10992	2.38	O	3.16	O	
12	85	3.42	BH	4.2	BH	
13	10853	3.79	BH	4.57	BH	
14	5055	0.96	O	1.74	O	
15	10908	1.74	O	2.52	O	
16	10837	2.99	N/AO	3.77	N/AO	
17	5144	1.99	O	2.77	O	
18	10005	2.68	N/AO	3.46	N/AO	
19	10269	4.65	H	5.43	H	
20	5108	4.59	H	5.37	H	
21	5247	1.52	O	2.3	O	
22	10418	1.29	O	2.07	O	
23	389	1.75	O	2.53	O	
24	5260	1.72	O	2.5	O	
25	36300	1.41	O	2.19	O	
26	5109	3.37	BH	4.15	BH	
27	10282	3.41	BH	4.19	BH	
28	10068	0.66	O	1.44	O	
29	36279	3.26	N/AO	4.04	N/AO	
30	10774	3.94	BH	4.72	BH	
31	35913	2.89	N/AO	3.67	N/AO	
32	10656	0.76	O	1.54	O	
33	36169	3.52	BH	4.3	BH	
34	5161	4.14	H	4.92	H	
35	10295	3.24	N/AO	4.02	N/AO	
36	10792	4.26	H	5.04	H	
37	36135	1.98	O	2.76	O	
38	10340	2.24	O	3.02	O	
39	36061	4.24	H	5.02	H	
40	1759	1.54	O	2.32	O	
41	12023	5.21	VH	5.99	VH	
42	1988	2.7	N/AO	3.48	N/AO	
43	1798	3.75	BH	4.53	BH	

44	11587	3.15	N/AO	3.93	N/AO	
45	5491	5.13	VH	5.91	VH	
46	11826	0.95	O	1.73	O	
47	11814	3.39	BH	4.17	BH	
48	11635	1.6	O	2.38	O	
49	11813	2.11	O	2.89	O	
50	11827	2.34	O	3.12	O	
51	12414	3.02	N/AO	3.80	N/AO	
52	12423	2.56	O	3.34	O	
53	12229	4.02	BH	4.80	BH	
54	22110	1.77	O	2.55	O	
55	22099	3.3	N/AO	4.08	N/AO	
56	22091	2.73	N/AO	3.51	N/AO	
57	22088	4.13	BH	4.91	H	↑ 1 category
58	22087	4.04	BH	4.82	BH	
59	22077	3.92	BH	4.70	BH	
60	22034	4.27	H	5.05	H	
61	22063	1.42	O	2.20	O	
62	22042	2.5	O	3.28	O	
63	22041	2.44	O	3.22	O	
64	22071	6.46	VH	7.24	VH	
65	22066	2.69	N/AO	3.47	N/AO	
66	22064	3.12	N/AO	3.90	N/AO	
67	22065	3.05	N/AO	3.83	N/AO	
68	22072	2.54	O	3.32	O	
69	12494	1.69	O	2.47	O	
70	12514	2.6	N/AO	3.38	N/AO	
71	12493	2.74	N/AO	3.52	N/AO	
72	5833	2.35	O	3.13	O	
73	12520	2.45	O	3.23	O	
74	12528	3.33	N/AO	4.11	N/AO	
75	12524	1.7	O	2.48	O	
76	22100	4.3	H	5.08	H	
77	22098	2.54	O	3.32	O	
78	22078	2.98	N/AO	3.76	N/AO	
79	22106	4.16	H	4.94	H	
80	22105	4.06	BH	4.84	BH	
81	12561	2.87	N/AO	3.65	N/AO	
82	12552	3.4	BH	4.18	BH	
83	22142	3.61	BH	4.39	BH	
84	22140	3.84	BH	4.62	BH	
85	22143	3.18	N/AO	3.96	N/AO	
86	22144	3.87	BH	4.65	BH	
87	22231			4.18	BH	Excluded sample
88	22191	3.45	BH	4.23	BH	
89	22152	1.47	O	2.25	O	
90	22155	4.55	H	5.33	H	
91	22157	2.6	N/AO	3.38	N/AO	
92	22207	7.37	VH	8.15	VH	
93	22208	4.33	H	5.11	H	

94	22230	2.01	O	2.79	O	
95	22206	5.82	VH	6.60	VH	
96	22189	3.36	BH	4.14	BH	
97	22237	4.54	H	5.32	H	
98	22218	3	N/AO	3.78	N/AO	
99	22188	2.28	O	3.06	O	
100	22167	2.82	N/AO	3.60	N/AO	
101	22164	3.93	BH	4.71	BH	
102	22168	4.76	H	5.54	H	
103	22171	2.02	O	2.80	O	
104	22187	1.59	O	2.37	O	
105	22186	1.48	O	2.26	O	
106	22184	4.42	H	5.20	H	
107	22267	3.5	BH	4.28	BH	
108	22334	1.91	O	2.69	O	
109	22352	2.96	N/AO	3.74	N/AO	
110	22323	2.86	N/AO	3.64	N/AO	
111	22322	2.97	N/AO	3.75	N/AO	
112	22351			5.18		Excluded sample
113	22360	2.41	O	3.19	O	
114	22335	5.62	VH	6.40	VH	
115	22307	1.7	O	2.48	O	
116	22308	3.54	BH	4.32	BH	
117	22320	3.01	N/AO	3.79	N/AO	
118	22318	3.09	N/AO	3.87	N/AO	
119	22330	4.37	H	5.15	H	
120	22327	2.74	N/AO	3.52	N/AO	
121	22328	4.3	H	5.08	H	
122	12577	4.47	H	5.25	H	
123	12589	2.84	N/AO	3.62	N/AO	

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high,

↑- increase, ↓ - decrease.

Appendix 9.15 A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) value HDLC/cLDLC ratio

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		HDLC/cLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
1	36301	3.99	R	0.266	R	
2	10038	5.08	R	0.250	R	
3	5231	4	R	0.218	R	
4	10309	3.79	R	0.145	R	
5	960	3.41	R	0.314	R	
6	10186	5.94	R	0.244	R	
7	5191	2.84	R	0.500	NR	C, R - NR
8	10775	4.07	R	0.531	NR	C, R - NR
9	10793	3.88	R	0.361	R	
10	10276	4.58	R	0.236	R	
11	10992	2.79	R	0.330	R	
12	85	3.79	R	0.240	R	
13	10853	3.99	R	0.308	R	
14	5055	1.51	NR	0.967	NR	
15	10908	2.22	NR	0.536	NR	
16	10837	2.6	R	0.362	R	
17	5144	2.68	R	0.642	NR	C, R - NR
18	10005	2.66	R	0.316	R	C, NR - R
19	10269	5.02	R	0.247	R	
20	5108	4.31	R	0.195	R	
21	5247	1.88	NR	0.734	NR	
22	10418	1.75	NR	0.640	NR	
23	389	2.21	NR	0.851	NR	
24	5260	1.78	NR	0.466	NR	
25	36300	1.91	NR	1.073	NR	
26	5109	3.79	R	0.459	NR	C, R - NR
27	10282	3.24	R	0.491	NR	C, R - NR
28	10068	1.16	NR	1.052	NR	
29	36279	3.5	R	0.400	NR	C, R - NR
30	10774	4.19	R	0.339	R	
31	35913	3.41	R	0.449	NR	C, R - NR
32	10656	1.26	NR	1.246	NR	
33	36169	3.73	R	0.370	R	
34	5161	4.42	R	0.181	R	
35	10295	3.52	R	0.366	R	
36	10792	4.51	R	0.302	R	
37	36135	2.48	NR	0.524	NR	
38	10340	2.49	NR	0.426	NR	
39	36061	4.4	R	0.252	R	

R – risk, NR – no risk, C – change

Appendix 9.15 (continued) A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) value and HDLC/cLDLC ratio

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		HDLC/cLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
40	1759	1.94	NR	0.747	NR	
41	12023	5.28	R	0.342	R	
42	1988	3.05	R	0.567	NR	C, R - NR
43	1798	4.05	R	0.338	R	
44	11587	3.11	R	0.273	R	
45	5491	5.46	R	0.293	R	
46	11826	1.34	NR	1.022	NR	
47	11814	3.62	R	0.309	R	
48	11635	2.09	NR	0.980	NR	
49	11813	2.59	R	0.618	NR	C, R - NR
50	11827	2.69	R	0.450	NR	C, R - NR
51	12414	3.51	R	0.362	R	
52	12423	2.14	NR	0.168	R	C, NR - R
53	12229	4.27	R	0.351	R	
54	22110	2.19	NR	0.530	NR	
55	22099	2.70	R	0.322	R	C, NR - R
56	22091	3.22	R	0.400	NR	C, R - NR
57	22088	4.21	R	0.271	R	
58	22087	4.18	R	0.275	R	
59	22077	3.38	R	0.260	R	
60	22034	3.14	R	0.290	R	
61	22063	1.94	NR	0.526	NR	
62	22042	2.80	R	0.343	R	
63	22041	2.54	NR	0.311	R	C, NR - R
64	22071	5.73	R	0.227	R	
65	22066	3.09	R	0.291	R	
66	22064	3.02	R	0.321	R	
67	22065	2.81	R	0.381	R	
68	22072	2.68	R	0.399	R	
69	12494	1.54	NR	0.675	NR	
70	12514	3.03	R	0.360	R	
71	12493	2.69	R	0.677	NR	C, R - NR
72	5833	2.61	R	0.678	NR	C, R - NR
73	12520	2.66	R	0.549	NR	C, R - NR
74	12528	3.51	R	0.362	R	
75	12524	1.82	NR	0.978	NR	
76	22100	4.55	R	0.207	R	
77	22098	2.64	R	0.777	NR	C, R - NR
78	22078	2.70	R	0.359	R	

R – risk, NR – no risk, C – change

Appendix 9.15 (continued) A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) value and HDLC/cLDLC ratio

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		HDLC/cLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
79	22106	4.47	R	0.477	NR	C, R - NR
80	22105	4.28	R	0.217	R	
81	12561	3.24	R	0.346	R	
82	12552	3.36	R	0.378	R	
83	22142	3.00	R	0.390	R	
84	22140	4.16	R	0.442	NR	C, R - NR
85	22143	3.35	R	0.325	R	
86	22144	4.24	R	0.292	R	
87	22231	1.04	O	0.433	NR	Excluded sample
88	22191	3.89	R	0.352	R	
89	22152	1.31	NR	0.908	NR	
90	22155	3.71	R	0.213	R	
91	22157	2.95	R	1.064	NR	C, R - NR
92	22207	6.78	R	0.090	R	
93	22208	4.30	R	0.344	R	
94	22230	2.20	NR	0.736	NR	
95	22206	6.04	R	0.293	R	
96	22189	3.64	R	0.401	NR	C, R - NR
97	22237	3.92	R	0.219	R	
98	22218	2.46	NR	0.557	NR	
99	22188	2.68	R	0.504	NR	C, R - NR
100	22167	3.25	R	0.492	NR	C, R - NR
101	22164	3.79	R	0.375	R	
102	22168	3.77	R	0.183	R	
103	22171	2.35	NR	0.319	R	C, NR - R
104	22187	2.09	NR	0.431	NR	
105	22186	2.05	NR	0.502	NR	
106	22184	4.32	R	0.234	R	
107	22267	3.15	R	0.257	R	
108	22334	2.48	NR	0.625	NR	
109	22352	3.06	R	0.261	R	
110	22323	2.99	R	0.405	NR	C, R - NR
111	22322	3.06	R	0.258	R	
112	22351	2.06		0.291		Excluded sample
113	22360	2.83	R	0.466	NR	C, R - NR
114	22335	5.73	R	0.140	R	
115	22307	1.94	NR	0.392	R	C, NR - R
116	22308	2.96	R	0.230	R	
117	22320	3.42	R	0.541	NR	C, R - NR

R – risk, NR – no risk, C – change

Appendix 9.15 (continued) A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) value and HDLC/cLDLC ratio

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		HDLC/cLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
118	22318	3.45	R	0.423	NR	C, R - NR
119	22330	4.07	R	0.273	R	
120	22327	2.84	R	0.257	R	
121	22328	3.48	R	0.287	R	
122	12577	4.13	R	0.262	R	
123	12589	3.21	R	0.364	R	

R – risk, NR – no risk, C – change

Appendix 9.16 A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) value and HDLC/dLDLC ratio

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		HDLC/dLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
1	36301	3.99	R	0.294	R	
2	10038	5.08	R	0.271	R	
3	5231	4	R	0.238	R	
4	10309	3.79	R	0.174	R	
5	960	3.41	R	0.331	R	
6	10186	5.94	R	0.257	R	
7	5191	2.84	R	0.566	NR	C, R - NR
8	10775	4.07	R	0.554	NR	C, R - NR
9	10793	3.88	R	0.379	R	
10	10276	4.58	R	0.262	R	
11	10992	2.79	R	0.349	R	
12	85	3.79	R	0.265	R	
13	10853	3.99	R	0.346	R	
14	5055	1.51	NR	0.924	NR	
15	10908	2.22	NR	0.559	NR	
16	10837	2.6	R	0.392	R	
17	5144	2.68	R	0.708	NR	C, R - NR
18	10005	2.66	R	0.351	R	
19	10269	5.02	R	0.267	R	
20	5108	4.31	R	0.208	R	
21	5247	1.88	NR	0.758	NR	
22	10418	1.75	NR	0.615	NR	
23	389	2.21	NR	0.821	NR	
24	5260	1.78	NR	0.456	NR	
25	36300	1.91	NR	1.041	NR	
26	5109	3.79	R	0.539	NR	C, R - NR
27	10282	3.24	R	0.582	NR	C, R - NR
28	10068	1.16	NR	1.162	NR	
29	36279	3.5	R	0.483	NR	C, R - NR
30	10774	4.19	R	0.374	R	
31	35913	3.41	R	0.515	NR	C, R - NR
32	10656	1.26	NR	1.236	NR	
33	36169	3.73	R	0.414	NR	C, R - NR
34	5161	4.42	R	0.206	R	
35	10295	3.52	R	0.442	NR	C, R - NR
36	10792	4.51	R	0.337	R	
37	36135	2.48	NR	0.583	NR	
38	10340	2.49	NR	0.480	NR	
39	36061	4.4	R	0.292	R	

R – risk, NR – no risk, C – change

Appendix 9.16 (continued) A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) value and HDLC/dLDLC ratio

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		HDLC/dLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
40	1759	1.94	NR	0.759	NR	
41	12023	5.28	R	0.358	R	
42	1988	3.05	R	0.584	NR	C, R - NR
43	1798	4.05	R	0.353	R	
44	11587	3.11	R	0.291	R	
45	5491	5.46	R	0.307	R	
46	11826	1.34	NR	1.030	NR	
47	11814	3.62	R	0.312	R	
48	11635	2.09	NR	0.972	NR	
49	11813	2.59	R	0.611	NR	C, R - NR
50	11827	2.69	R	0.481	NR	C, R - NR
51	12414	3.51	R	0.350	R	
52	12423	2.14	NR	0.269	R	C, NR - R
53	12229	4.27	R	0.349	R	
54	22110	2.19	NR	0.560	NR	
55	22099	2.70	R	0.286	R	C, NR - R
56	22091	3.22	R	0.426	NR	C, R - NR
57	22088	4.21	R	0.273	R	
58	22087	4.18	R	0.274	R	
59	22077	3.38	R	0.260	R	
60	22034	3.14	R	0.270	R	C, NR - R
61	22063	1.94	NR	0.583	NR	
62	22042	2.80	R	0.400	NR	C, R - NR
63	22041	2.54	NR	0.348	R	C, NR - R
64	22071	5.73	R	0.246	R	
65	22066	3.09	R	0.314	R	
66	22064	3.02	R	0.324	R	
67	22065	2.81	R	0.398	R	
68	22072	2.68	R	0.420	NR	C, R - NR
69	12494	1.54	NR	0.693	NR	
70	12514	3.03	R	0.355	R	
71	12493	2.69	R	0.703	NR	C, R - NR
72	5833	2.61	R	0.678	NR	C, R - NR
73	12520	2.66	R	0.512	NR	C, R - NR
74	12528	3.51	R	0.378	R	
75	12524	1.82	NR	0.886	NR	
76	22100	4.55	R	0.210	R	
77	22098	2.64	R	0.745	NR	C, R - NR
78	22078	2.70	R	0.346	R	

R – risk, NR – no risk, C – change

Appendix 9.16 (continued) A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) value and HDLC/dLDLC ratio

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		HDLC/dLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
79	22106	4.47	R	0.447	NR	C, R - NR
80	22105	4.28	R	0.226	R	
81	12561	3.24	R	0.396	NR	C, R - NR
82	12552	3.36	R	0.452	NR	C, R - NR
83	22142	3.00	R	0.443	NR	C, R - NR
84	22140	4.16	R	0.559	NR	C, R - NR
85	22143	3.35	R	0.398	R	
86	22144	4.24	R	0.359	R	
87	22231	1.04	O	0.344	R	Excluded sample
88	22191	3.89	R	0.413	NR	C, R - NR
89	22152	1.31	NR	0.944	NR	
90	22155	3.71	R	0.231	R	
91	22157	2.95	R	1.241	NR	C, R - NR
92	22207	6.78	R	0.107	R	
93	22208	4.30	R	0.399	R	
94	22230	2.20	NR	0.814	NR	
95	22206	6.04	R	0.348	R	
96	22189	3.64	R	0.471	NR	C, R - NR
97	22237	3.92	R	0.279	R	
98	22218	2.46	NR	0.578	NR	
99	22188	2.68	R	0.577	NR	C, R - NR
100	22167	3.25	R	0.576	NR	C, R - NR
101	22164	3.79	R	0.434	NR	C, R - NR
102	22168	3.77	R	0.209	R	
103	22171	2.35	NR	0.385	R	C, NR - R
104	22187	2.09	NR	0.520	NR	
105	22186	2.05	NR	0.599	NR	
106	22184	4.32	R	0.284	R	
107	22267	3.15	R	0.279	R	
108	22334	2.48	NR	0.718	NR	
109	22352	3.06	R	0.310	R	
110	22323	2.99	R	0.465	NR	C, R - NR
111	22322	3.06	R	0.312	R	
112	22351	2.06		0.208		Excluded sample
113	22360	2.83	R	0.522	NR	C, R - NR
114	22335	5.73	R	0.162	R	
115	22307	1.94	NR	0.475	NR	
116	22308	2.96	R	0.248	R	
117	22320	3.42	R	0.636	NR	C, R - NR

R – risk, NR – no risk, C – change

Appendix 9.16 (continued) A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) value and HDLC/dLDLC ratio

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		HDLC/dLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
118	22318	3.45	R	0.477	NR	C, R - NR
119	22330	4.07	R	0.303	R	
120	22327	2.84	R	0.312	R	
121	22328	3.48	R	0.284	R	
122	12577	4.13	R	0.308	R	
123	12589	3.21	R	0.351	R	

R – risk, NR – no risk, C – change

Appendix 9.17 A comparison of CV risk establishment using the Roche direct LDLC (dLDLC) value and HDLC/dLDLC ratio and their risk categories

Sequential number	Lab ID	Roche direct LDLC (dLDLC)		HDLC/dLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
1	36301	3.6	R	0.294	R	
2	10038	4.69	R	0.271	R	
3	5231	3.65	R	0.238	R	
4	10309	3.17	R	0.174	R	
5	960	3.23	R	0.331	R	
6	10186	5.65	R	0.257	R	
7	5191	2.51	NR	0.566	NR	
8	10775	3.9	R	0.554	NR	C,R - NR
9	10793	3.69	R	0.379	R	
10	10276	4.12	R	0.262	R	
11	10992	2.63	R	0.349	R	
12	85	3.44	R	0.265	R	
13	10853	3.55	R	0.346	R	
14	5055	1.58	NR	0.924	NR	
15	10908	2.13	NR	0.559	NR	
16	10837	2.4	NR	0.392	R	C,NR - R
17	5144	2.43	NR	0.708	NR	
18	10005	2.39	NR	0.351	R	C,NR - R
19	10269	4.65	R	0.267	R	
20	5108	4.03	R	0.208	R	
21	5247	1.82	NR	0.758	NR	
22	10418	1.82	NR	0.615	NR	
23	389	2.29	NR	0.821	NR	
24	5260	1.82	NR	0.456	NR	
25	36300	1.97	NR	1.041	NR	
26	5109	3.23	R	0.539	NR	C, R - NR
27	10282	2.73	R	0.582	NR	C, R - NR
28	10068	1.05	NR	1.162	NR	
29	36279	2.9	R	0.483	NR	C, R - NR
30	10774	3.8	R	0.374	R	
31	35913	2.97	R	0.515	NR	C, R - NR
32	10656	1.27	NR	1.236	NR	
33	36169	3.33	R	0.414	NR	C, R - NR
34	5161	3.88	R	0.206	R	
35	10295	2.92	R	0.442	NR	C, R - NR
36	10792	4.04	R	0.337	R	
37	36135	2.23	NR	0.583	NR	
38	10340	2.21	NR	0.480	NR	
39	36061	3.8	R	0.292	R	

R – risk, NR – no risk, C – change

Appendix 9.17 (continued) A comparison of CV risk establishment using the Roche direct LDLC (dLDLC) value and HDLC/dLDLC ratio and their risk categories

Sequential number	Lab ID	Roche direct LDLC (dLDLC)		HDLC/dLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
40	1759	1.91	NR	0.759	NR	
41	12023	5.05	R	0.358	R	
42	1988	2.96	R	0.584	NR	C, R - NR
43	1798	3.88	R	0.353	R	
44	11587	2.92	R	0.291	R	
45	5491	5.21	R	0.307	R	
46	11826	1.33	NR	1.030	NR	
47	11814	3.59	R	0.312	R	
48	11635	2.11	NR	0.972	NR	
49	11813	2.62	R	0.611	NR	C, R - NR
50	11827	2.68	R	0.481	NR	C, R - NR
51	12414	3.63	R	0.350	R	
52	12423	1.34	NR	0.269	R	C,NR - R
53	12229	4.30	R	0.349	R	
54	22110	2.07	NR	0.560	NR	
55	22099	3.04	R	0.286	R	
56	22091	3.03	R	0.426	NR	C, R - NR
57	22088	4.18	R	0.273	R	
58	22087	4.20	R	0.274	R	
59	22077	3.39	R	0.260	R	
60	22034	3.37	R	0.270	R	
61	22063	1.75	NR	0.583	NR	
62	22042	2.40	NR	0.400	NR	
63	22041	2.27	NR	0.348	R	C,NR - R
64	22071	5.29	R	0.246	R	
65	22066	2.87	R	0.314	R	
66	22064	2.99	R	0.324	R	
67	22065	2.69	R	0.398	R	
68	22072	2.55	NR	0.420	NR	
69	12494	1.50	NR	0.693	NR	
70	12514	3.07	R	0.355	R	
71	12493	2.59	R	0.703	NR	C, R - NR
72	5833	2.61	R	0.678	NR	C, R - NR
73	12520	2.85	R	0.512	NR	C, R - NR
74	12528	3.36	R	0.378	R	
75	12524	2.01	NR	0.886	NR	
76	22100	4.48	R	0.210	R	
77	22098	2.75	R	0.745	NR	C, R - NR
78	22078	2.80	R	0.346	R	

R – risk, NR – no risk, C – change

Appendix 9.17 (continued) A comparison of CV risk establishment using the Roche direct LDLC (dLDLC) value and HDLC/dLDLC ratio and their risk categories

Sequential number	Lab ID	Roche direct LDLC (dLDLC)		HDLC/dLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
79	22106	4.76	R	0.447	NR	C,R - NR
80	22105	4.12	R	0.226	R	
81	12561	2.83	R	0.396	R	
82	12552	2.81	R	0.452	NR	C, R - NR
83	22142	2.64	R	0.443	NR	C, R - NR
84	22140	3.29	R	0.559	NR	C, R - NR
85	22143	2.74	R	0.398	R	
86	22144	3.45	R	0.359	R	
87	22231	1.31		0.344		Excluded sample
88	22191	3.32	R	0.413	NR	C, R - NR
89	22152	1.26	NR	0.944	NR	
90	22155	3.42	R	0.231	R	
91	22157	2.53	NR	1.241	NR	
92	22207	5.68	R	0.107	R	
93	22208	3.71	R	0.399	R	
94	22230	1.99	NR	0.814	NR	
95	22206	5.08	R	0.348	R	
96	22189	3.10	R	0.471	NR	C, R - NR
97	22237	3.08	R	0.279	R	
98	22218	2.37	NR	0.578	NR	
99	22188	2.34	NR	0.577	NR	
100	22167	2.78	R	0.576	NR	C, R - NR
101	22164	3.27	R	0.434	NR	C, R - NR
102	22168	3.30	R	0.209	R	
103	22171	1.95	NR	0.385	R	C,NR - R
104	22187	1.73	NR	0.520	NR	
105	22186	1.72	NR	0.599	NR	
106	22184	3.56	R	0.284	R	
107	22267	2.90	R	0.279	R	
108	22334	2.16	NR	0.718	NR	
109	22352	2.58	NR	0.310	R	C,NR - R
110	22323	2.60	R	0.465	NR	C, R - NR
111	22322	2.53	NR	0.312	R	C,NR - R
112	22351	2.88		0.208		Excluded sample
113	22360	2.53	NR	0.522	NR	
114	22335	4.95	R	0.162	R	
115	22307	1.60	NR	0.475	NR	
116	22308	2.74	R	0.248	R	
117	22320	2.91	R	0.636	NR	C, R - NR

R – risk, NR – no risk, C – change

Appendix 9.17 (continued) A comparison of CV risk estimation using the Roche direct LDLC (dLDLC) value and HDLC/dLDLC ratio

Sequential number	Lab ID	Roche direct LDLC (dLDLC)		HDLC/dLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
118	22318	3.06	R	0.477	NR	C, R - NR
119	22330	3.66	R	0.303	R	
120	22327	2.34	NR	0.312	R	C,NR - R
121	22328	3.52	R	0.284	R	
122	12577	3.51	R	0.308	R	
123	12589	3.33	R	0.351	R	

R – risk, NR – no risk, C – change

Appendix 9.18 A comparison of CV risk estimation using the Roche direct LDLC (dLDLC) value and HDLC/cLDLC ratio

Sequential number	Lab ID	Roche directLDLC (dLDLC)		HDLC/cLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
1	36301	3.6	R	0.266	R	
2	10038	4.69	R	0.250	R	
3	5231	3.65	R	0.218	R	
4	10309	3.17	R	0.145	R	
5	960	3.23	R	0.314	R	
6	10186	5.65	R	0.244	R	
7	5191	2.51	NR	0.500	NR	
8	10775	3.9	R	0.531	NR	C,R - NR
9	10793	3.69	R	0.361	R	
10	10276	4.12	R	0.236	R	
11	10992	2.63	R	0.330	R	
12	85	3.44	R	0.240	R	
13	10853	3.55	R	0.308	R	
14	5055	1.58	NR	0.967	NR	
15	10908	2.13	NR	0.536	NR	
16	10837	2.4	NR	0.362	R	C,NR - R
17	5144	2.43	NR	0.642	NR	
18	10005	2.39	NR	0.316	R	C,NR - R
19	10269	4.65	R	0.247	R	
20	5108	4.03	R	0.195	R	
21	5247	1.82	NR	0.734	NR	
22	10418	1.82	NR	0.640	NR	
23	389	2.29	NR	0.851	NR	
24	5260	1.82	NR	0.466	NR	
25	36300	1.97	NR	1.073	NR	
26	5109	3.23	R	0.459	NR	C,R - NR
27	10282	2.73	R	0.491	NR	C,R - NR
28	10068	1.05	NR	1.052	NR	
29	36279	2.9	R	0.400	NR	C,R - NR
30	10774	3.8	R	0.339	R	
31	35913	2.97	R	0.449	NR	C,R - NR
32	10656	1.27	NR	1.246	NR	
33	36169	3.33	R	0.370	R	
34	5161	3.88	R	0.181	R	
35	10295	2.92	R	0.366	R	
36	10792	4.04	R	0.302	R	
37	36135	2.23	NR	0.524	NR	
38	10340	2.21	NR	0.426	NR	
39	36061	3.8	R	0.252	R	

BH – borderline high, H – high, N/AO – near/above optimal, O – optimal, VH – very high,

R – risk, NR – no risk, C – change, NC – no change

Appendix 9.18 (continued) A comparison of CV risk establishment using the Roche direct LDLC (dLDLC) value and HDLC/cLDLC ratio and their risk categories

Sequential number	Lab ID	Roche directLDLC (dLDLC)		HDLC/cLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
40	1759	1.91	NR	0.747	NR	
41	12023	5.05	R	0.342	R	
42	1988	2.96	R	0.567	NR	C,R - NR
43	1798	3.88	R	0.338	R	
44	11587	2.92	R	0.273	R	
45	5491	5.21	R	0.293	R	
46	11826	1.33	NR	1.022	NR	
47	11814	3.59	R	0.309	R	
48	11635	2.11	NR	0.980	NR	
49	11813	2.62	R	0.618	NR	C,R - NR
50	11827	2.68	R	0.450	NR	C,R - NR
51	12414	3.63	R	0.362	R	
52	12423	1.34	NR	0.168	R	C,NR - R
53	12229	4.30	R	0.351	R	
54	22110	2.07	NR	0.530	NR	
55	22099	3.04	R	0.322	R	
56	22091	3.03	R	0.400	NR	C,R - NR
57	22088	4.18	R	0.271	R	
58	22087	4.20	R	0.275	R	
59	22077	3.39	R	0.260	R	
60	22034	3.37	R	0.290	R	
61	22063	1.75	NR	0.526	NR	
62	22042	2.40	NR	0.343	R	C,NR - R
63	22041	2.27	NR	0.311	R	C,NR - R
64	22071	5.29	R	0.227	R	
65	22066	2.87	R	0.291	R	
66	22064	2.99	R	0.321	R	
67	22065	2.69	R	0.381	R	
68	22072	2.55	NR	0.399	R	C,NR - R
69	12494	1.50	NR	0.675	NR	
70	12514	3.07	R	0.360	R	
71	12493	2.59	R	0.677	NR	C,R - NR
72	5833	2.61	R	0.678	NR	C,R - NR
73	12520	2.85	R	0.549	NR	C,R - NR
74	12528	3.36	R	0.362	R	
75	12524	2.01	NR	0.978	NR	
76	22100	4.48	R	0.207	R	
77	22098	2.75	R	0.777	NR	C,R - NR
78	22078	2.80	R	0.359	R	

R – risk, NR – no risk, C – change

Appendix 9.18 (continued) A comparison of CV risk estimation using the Roche direct LDLC (dLDLC) and HDLC/cLDLC ratio

Sequential number	Lab ID	Roche directLDLC (dLDLC)		HDLC/cLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
79	22106	4.76	R	0.477	NR	C,R - NR
80	22105	4.12	R	0.217	R	
81	12561	2.83	R	0.346	R	
82	12552	2.81	R	0.378	R	
83	22142	2.64	R	0.390	R	
84	22140	3.29	R	0.442	NR	C,R - NR
85	22143	2.74	R	0.325	R	
86	22144	3.45	R	0.292	R	
87	22231	1.31		0.433		Excluded sample
88	22191	3.32	R	0.352	R	
89	22152	1.26	NR	0.908	NR	
90	22155	3.42	R	0.213	R	
91	22157	2.53	NR	1.064	NR	
92	22207	5.68	R	0.090	R	
93	22208	3.71	R	0.344	R	
94	22230	1.99	NR	0.736	NR	
95	22206	5.08	R	0.293	R	
96	22189	3.10	R	0.401	NR	C,R - NR
97	22237	3.08	R	0.219	R	
98	22218	2.37	NR	0.557	NR	
99	22188	2.34	NR	0.504	NR	
100	22167	2.78	R	0.492	NR	C,R - NR
101	22164	3.27	R	0.375	R	
102	22168	3.30	R	0.183	R	
103	22171	1.95	NR	0.319	R	C,NR - R
104	22187	1.73	NR	0.431	NR	
105	22186	1.72	NR	0.502	NR	
106	22184	3.56	R	0.234	R	
107	22267	2.90	R	0.257	R	
108	22334	2.16	NR	0.625	NR	
109	22352	2.58	NR	0.261	R	C,NR - R
110	22323	2.60	R	0.405	NR	C,R - NR
111	22322	2.53	NR	0.258	R	C,NR - R
112	22351	2.88		0.291		Excluded sample
113	22360	2.53	NR	0.466	NR	
114	22335	4.95	R	0.140	R	
115	22307	1.60	NR	0.392	R	C,NR - R
116	22308	2.74	R	0.230	R	
117	22320	2.91	R	0.541	NR	C,R - NR

R – risk, NR – no risk, C – change

Appendix 9.18 (continued) A comparison of CV risk estimation using the Roche direct LDLC (dLDLC) and HDLC/cLDLC ratio

Sequential number	Lab ID	Roche directLDLC (dLDLC)		HDLC/cLDLC		Change(C) or no change (NC) in risk status
		value	risk category	value	risk (R) or no risk(NR)	
118	22318	3.06	R	0.423	NR	C,R - NR
119	22330	3.66	R	0.273	R	
120	22327	2.34	NR	0.257	R	C,NR - R
121	22328	3.52	R	0.287	R	
122	12577	3.51	R	0.262	R	
123	12589	3.33	R	0.364	R	

R – risk, NR – no risk, C – change

Appendix 9.19 A comparison of CV risk establishment using the HDLC/cLDLC and the HDLC/dLDLC ratio and their risk categories

Sequential number	Lab ID	HDLC/cLDLC		HDLC/dLDLC		Change(C) or no change (NC) in risk category
		value	risk category	value	risk category	
1	36301	0.266	R	0.294	R	
2	10038	0.250	R	0.271	R	
3	5231	0.218	R	0.238	R	
4	10309	0.145	R	0.174	R	
5	960	0.314	R	0.331	R	
6	10186	0.244	R	0.257	R	
7	5191	0.500	NR	0.566	NR	
8	10775	0.531	NR	0.554	NR	
9	10793	0.361	R	0.379	R	
10	10276	0.236	R	0.262	R	
11	10992	0.330	R	0.349	R	
12	85	0.240	R	0.265	R	
13	10853	0.308	R	0.346	R	
14	5055	0.967	NR	0.924	NR	
15	10908	0.536	NR	0.559	NR	
16	10837	0.362	R	0.392	R	
17	5144	0.642	NR	0.708	NR	
18	10005	0.316	R	0.351	R	
19	10269	0.247	R	0.267	R	
20	5108	0.195	R	0.208	R	
21	5247	0.734	NR	0.758	NR	
22	10418	0.640	NR	0.615	NR	
23	389	0.851	NR	0.821	NR	
24	5260	0.466	NR	0.456	NR	
25	36300	1.073	NR	1.041	NR	
26	5109	0.459	NR	0.539	NR	
27	10282	0.491	NR	0.582	NR	
28	10068	1.052	NR	1.162	NR	
29	36279	0.400	NR	0.483	NR	
30	10774	0.339	R	0.374	R	
31	35913	0.449	NR	0.515	NR	
32	10656	1.246	NR	1.236	NR	
33	36169	0.370	R	0.414	NR	C,R - NR
34	5161	0.181	R	0.206	R	
35	10295	0.366	R	0.442	NR	C,R - NR
36	10792	0.302	R	0.337	R	
37	36135	0.524	NR	0.583	NR	
38	10340	0.426	NR	0.480	NR	
39	36061	0.252	R	0.292	R	

R – risk, NR – no risk, C – change

Appendix 9.19 (continued) A comparison of CV risk estimation using the HDLC/cLDLC and the HDLC/dLDLC ratios

Sequential number	Lab ID	HDLC/cLDLC		HDLC/dLDLC		Change(C) or no change (NC) in risk category
		value	risk category	value	risk category	
40	1759	0.747	NR	0.759	NR	
41	12023	0.342	R	0.358	R	
42	1988	0.567	NR	0.584	NR	
43	1798	0.338	R	0.353	R	
44	11587	0.273	R	0.291	R	
45	5491	0.293	R	0.307	R	
46	11826	1.022	NR	1.030	NR	
47	11814	0.309	R	0.312	R	
48	11635	0.980	NR	0.972	NR	
49	11813	0.618	NR	0.611	NR	
50	11827	0.450	NR	0.481	NR	
51	12414	0.362	R	0.350	R	
52	12423	0.168	R	0.269	R	
53	12229	0.351	R	0.349	R	
54	22110	0.530	NR	0.560	NR	
55	22099	0.322	R	0.286	R	
56	22091	0.400	NR	0.426	NR	
57	22088	0.271	R	0.273	R	
58	22087	0.275	R	0.274	R	
59	22077	0.260	R	0.260	R	
60	22034	0.290	R	0.270	R	
61	22063	0.526	NR	0.583	NR	
62	22042	0.343	R	0.400	NR	C,R - NR
63	22041	0.311	R	0.348	R	
64	22071	0.227	R	0.246	R	
65	22066	0.291	R	0.314	R	
66	22064	0.321	R	0.324	R	
67	22065	0.381	R	0.398	R	
68	22072	0.399	R	0.420	NR	C,R - NR
69	12494	0.675	NR	0.693	NR	
70	12514	0.360	R	0.355	R	
71	12493	0.677	NR	0.703	NR	
72	5833	0.678	NR	0.678	NR	
73	12520	0.549	NR	0.512	NR	
74	12528	0.362	R	0.378	R	
75	12524	0.978	NR	0.886	NR	
76	22100	0.207	R	0.210	R	
77	22098	0.777	NR	0.745	NR	
78	22078	0.359	R	0.346	R	

R – risk, NR – no risk, C – change, NC – no change

Appendix 9.19 (continued) A comparison of CV risk estimation using the HDLC/cLDLC and the HDLC/dLDLC ratios

Sequential number	Lab ID	HDLC/cLDLC		HDLC/dLDLC		Change(C) or no change (NC) in risk category
		value	risk category	value	risk category	
79	22106	0.477	NR	0.447	NR	
80	22105	0.217	R	0.226	R	
81	12561	0.346	R	0.396	R	
82	12552	0.378	R	0.452	NR	C,R - NR
83	22142	0.390	R	0.443	NR	C,R - NR
84	22140	0.442	NR	0.559	NR	
85	22143	0.325	R	0.398	R	
86	22144	0.292	R	0.359	R	
87	22231	0.433		0.344		Excluded sample
88	22191	0.352	R	0.413	NR	C,R - NR
89	22152	0.908	NR	0.944	NR	
90	22155	0.213	R	0.231	R	
91	22157	1.064	NR	1.241	NR	
92	22207	0.090	R	0.107	R	
93	22208	0.344	R	0.399	R	
94	22230	0.736	NR	0.814	NR	
95	22206	0.293	R	0.348	R	
96	22189	0.401	NR	0.471	NR	
97	22237	0.219	R	0.279	R	
98	22218	0.557	NR	0.578	NR	
99	22188	0.504	NR	0.577	NR	
100	22167	0.492	NR	0.576	NR	
101	22164	0.375	R	0.434	NR	C,R - NR
102	22168	0.183	R	0.209	R	
103	22171	0.319	R	0.385	R	
104	22187	0.431	NR	0.520	NR	
105	22186	0.502	NR	0.599	NR	
106	22184	0.234	R	0.284	R	
107	22267	0.257	R	0.279	R	
108	22334	0.625	NR	0.718	NR	
109	22352	0.261	R	0.310	R	
110	22323	0.405	NR	0.465	NR	
111	22322	0.258	R	0.312	R	
112	22351	0.291		0.208		Excluded sample
113	22360	0.466	NR	0.522	NR	
114	22335	0.140	R	0.162	R	
115	22307	0.392	R	0.475	NR	C,R - NR
116	22308	0.230	R	0.248	R	
117	22320	0.541	NR	0.636	NR	

R – risk, NR – no risk, C – change, NC – no change

Appendix 9.19 (continued) A comparison of CV risk estimation using the HDLC/cLDLC and the HDLC/dLDLC ratios

Sequential number	Lab ID	HDLC/cLDLC		HDLC/dLDLC		Change(C) or no change (NC) in risk category
		value	risk category	value	risk category	
118	22318	0.423	NR	0.477	NR	
119	22330	0.273	R	0.303	R	
120	22327	0.257	R	0.312	R	
121	22328	0.287	R	0.284	R	
122	12577	0.262	R	0.308	R	
123	12589	0.364	R	0.351	R	

R – risk, NR – no risk, C – change

Appendix 9.20 A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) and sdLDLC

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		sdLDLC		Change in risk category/status
		value	risk category	value	risk (R) or no risk(NR)	
1	36301	3.99	R	0.819	R	
2	10038	5.08	R	1.589	R	
3	5231	4	R	1.046	R	
4	10309	3.79	R	1.400	R	
5	960	3.41	R	0.947	R	
6	10186	5.94	R	1.521	R	
7	5191	2.84	R	0.548	R	
8	10775	4.07	R	0.829	R	
9	10793	3.88	R	1.005	R	
10	10276	4.58	R	1.092	R	
11	10992	2.79	R	0.588	R	
12	85	3.79	R	0.800	R	
13	10853	3.99	R	0.913	R	
14	5055	1.51	NR	0.260	NR	
15	10908	2.22	NR	0.424	NR	
16	10837	2.6	R	0.980	R	
17	5144	2.68	R	0.360	NR	C, R - NR
18	10005	2.66	R	0.754	R	
19	10269	5.02	R	1.121	R	
20	5108	4.31	R	1.342	R	
21	5247	1.88	NR	0.414	NR	
22	10418	1.75	NR	0.376	NR	
23	389	2.21	NR	0.504	R	C, NR - R
24	5260	1.78	NR	0.602	R	C, NR - R
25	36300	1.91	NR	0.392	NR	
26	5109	3.79	R	0.683	R	
27	10282	3.24	R	0.896	R	
28	10068	1.16	NR	0.120	NR	
29	36279	3.5	R	0.692	R	
30	10774	4.19	R	0.959	R	
31	35913	3.41	R	0.574	R	
32	10656	1.26	NR	0.197	NR	
33	36169	3.73	R	0.855	R	
34	5161	4.42	R	0.941	R	
35	10295	3.52	R	0.675	R	
36	10792	4.51	R	1.011	R	
37	36135	2.48	NR	0.415	NR	
38	10340	2.49	NR	0.549	R	C, NR - R
39	36061	4.4	R	0.980	R	

R – risk, NR – no risk, C – change

Appendix 9.20 (continued) A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) and sdLDLC

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		SdLDLC		Change in risk category/status
		value	risk category	value	risk (R) or no risk(NR)	
40	1759	1.94	NR	0.420	NR	
41	12023	5.28	R	1.421	R	
42	1988	3.05	R	0.721	R	
43	1798	4.05	R	0.985	R	
44	11587	3.11	R	0.920	R	
45	5491	5.46	R	1.312	R	
46	11826	1.34	NR	0.273	NR	
47	11814	3.62	R	0.968	R	
48	11635	2.09	NR	0.429	NR	
49	11813	2.59	R	0.573	R	
50	11827	2.69	R	0.658	R	
51	12414	3.51	R	0.851	R	
52	12423	2.14	NR	0.624	R	C, NR - R
53	12229	4.27	R	1.156	R	
54	22110	2.19	NR	0.438	NR	
55	22099	2.70	R	1.353	R	
56	22091	3.22	R	0.644	R	
57	22088	4.21	R	1.212	R	
58	22087	4.18	R	1.191	R	
59	22077	3.38	R	1.363	R	
60	22034	3.14	R	1.730	R	
61	22063	1.94	NR	0.284	NR	
62	22042	2.80	R	0.554	R	
63	22041	2.54	NR	0.653	R	C, NR - R
64	22071	5.73	R	1.914	R	
65	22066	3.09	R	0.649	R	
66	22064	3.02	R	0.997	R	
67	22065	2.81	R	0.984	R	
68	22072	2.68	R	0.726	R	
69	12494	1.54	NR	0.625	R	C, NR - R
70	12514	3.03	R	0.724	R	
71	12493	2.69	R	0.850	R	
72	5833	2.61	R	0.692	R	
73	12520	2.66	R	0.814	R	
74	12528	3.51	R	0.917	R	
75	12524	1.82	NR	0.640	R	C, NR - R
76	22100	4.55	R	1.189	R	
77	22098	2.64	R	0.838	R	
78	22078	2.70	R	1.069	R	

R – risk, NR – no risk, C – change

Appendix 9.20 (continued) A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) and sdLDLC

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		SdLDLC		Change in risk category/status
		value	risk category	value	risk (R) or no risk(NR)	
79	22106	4.47	R	1.283	R	
80	22105	4.28	R	1.096	R	
81	12561	3.24	R	0.627	R	
82	12552	3.36	R	0.837	R	
83	22142	3.00	R	1.147	R	
84	22140	4.16	R	0.711	R	
85	22143	3.35	R	0.688	R	
86	22144	4.24	R	0.737	R	
87	22231	1.04		1.891		Excluded sample
88	22191	3.89	R	0.694	R	
89	22152	1.31	NR	0.565	R	C, NR - R
90	22155	3.71	R	1.499	R	
91	22157	2.95	R	0.557	R	
92	22207	6.78	R	1.840	R	
93	22208	4.30	R	1.066	R	
94	22230	2.20	NR	0.535	R	C, NR - R
95	22206	6.04	R	1.234	R	
96	22189	3.64	R	0.731	R	
97	22237	3.92	R	1.199	R	
98	22218	2.46	NR	1.075	R	C, NR - R
99	22188	2.68	R	0.489	NR	C, R - NR
100	22167	3.25	R	0.570	R	
101	22164	3.79	R	1.022	R	
102	22168	3.77	R	1.526	R	
103	22171	2.35	NR	0.416	NR	
104	22187	2.09	NR	0.265	NR	
105	22186	2.05	NR	0.227	NR	
106	22184	4.32	R	1.041	R	
107	22267	3.15	R	1.084	R	
108	22334	2.48	NR	0.346	NR	
109	22352	3.06	R	0.705	R	
110	22323	2.99	R	0.706	R	
111	22322	3.06	R	0.690	R	
112	22351	2.06		2.382		Excluded sample
113	22360	2.83	R	0.534	R	
114	22335	5.73	R	1.290	R	
115	22307	1.94	NR	0.383	NR	
116	22308	2.96	R	1.178	R	
117	22320	3.42	R	0.611	R	

R – risk, NR – no risk, C – change

Appendix 9.20 (continued) A comparison of CV risk estimation using the Friedewald calculated LDLC (cLDLC) and sdLDLC

Sequential number	Lab ID	Friedewald LDLC (cLDLC)		SdLDLC		Change in risk category/status
		value	risk category	value	risk (R) or no risk(NR)	
118	22318	3.45	R	0.697	R	
119	22330	4.07	R	1.235	R	
120	22327	2.84	R	0.638	R	
121	22328	3.48	R	1.563	R	
122	12577	4.13	R	1.186	R	
123	12589	3.21	R	0.840	R	

R – risk, NR – no risk, C – change

Appendix 9.21 A comparison of CV risk estimation using the Roche diagnostic direct LDLC (dLDLC) and the sdLDLC

Sequential number	Lab ID	Roche Diagnostics LDLC (dLDLC)		SdLDLC		Change in risk category/status
		value	risk category	value	risk (R) or no risk (NR)	
1	36301	3.6	R	0.819	R	
2	10038	4.69	R	1.589	R	
3	5231	3.65	R	1.046	R	
4	10309	3.17	R	1.400	R	
5	960	3.23	R	0.947	R	
6	10186	5.65	R	1.521	R	
7	5191	2.51	NR	0.548	R	C, NR - R
8	10775	3.9	R	0.829	R	
9	10793	3.69	R	1.005	R	
10	10276	4.12	R	1.092	R	
11	10992	2.63	R	0.588	R	
12	85	3.44	R	0.800	R	
13	10853	3.55	R	0.913	R	
14	5055	1.58	NR	0.260	NR	
15	10908	2.13	NR	0.424	NR	
16	10837	2.4	NR	0.980	R	C, NR - R
17	5144	2.43	NR	0.360	NR	
18	10005	2.39	NR	0.754	R	C, NR - R
19	10269	4.65	R	1.121	R	
20	5108	4.03	R	1.342	R	
21	5247	1.82	NR	0.414	NR	
22	10418	1.82	NR	0.376	NR	
23	389	2.29	NR	0.504	R	C, NR - R
24	5260	1.82	NR	0.602	R	C, NR - R
25	36300	1.97	NR	0.392	NR	
26	5109	3.23	R	0.683	R	
27	10282	2.73	R	0.896	R	
28	10068	1.05	NR	0.120	NR	
29	36279	2.9	R	0.692	R	
30	10774	3.8	R	0.959	R	
31	35913	2.97	R	0.574	R	
32	10656	1.27	NR	0.197	NR	
33	36169	3.33	R	0.855	R	
34	5161	3.88	R	0.941	R	
35	10295	2.92	R	0.675	R	
36	10792	4.04	R	1.011	R	
37	36135	2.23	NR	0.415	NR	
38	10340	2.21	NR	0.549	R	C, NR - R
39	36061	3.8	R	0.980	R	

R – risk, NR – no risk, C – change

Appendix 9.21 (continued) A comparison of CV risk estimation using the Roche diagnostic direct LDLC (dLDLC) and the sdLDLC

Sequential number	Lab ID	Roche Diagnostics LDLC (dLDLC)		SdLDLC		Change in risk category/status
		value	risk category	value	risk (R) or no risk (NR)	
40	1759	1.91	NR	0.420	NR	
41	12023	5.05	R	1.421	R	
42	1988	2.96	R	0.721	R	
43	1798	3.88	R	0.985	R	
44	11587	2.92	R	0.920	R	
45	5491	5.21	R	1.312	R	
46	11826	1.33	NR	0.273	NR	
47	11814	3.59	R	0.968	R	
48	11635	2.11	NR	0.429	NR	
49	11813	2.62	R	0.573	R	
50	11827	2.68	R	0.658	R	
51	12414	3.63	R	0.851	R	
52	12423	1.34	NR	0.624	R	C, NR - R
53	12229	4.30	R	1.156	R	
54	22110	2.07	NR	0.438	NR	
55	22099	3.04	R	1.353	R	
56	22091	3.03	R	0.644	R	
57	22088	4.18	R	1.212	R	
58	22087	4.20	R	1.191	R	
59	22077	3.39	R	1.363	R	
60	22034	3.37	R	1.730	R	
61	22063	1.75	NR	0.284	NR	
62	22042	2.40	NR	0.554	R	C, NR - R
63	22041	2.27	NR	0.653	R	C, NR - R
64	22071	5.29	R	1.914	R	
65	22066	2.87	R	0.649	R	
66	22064	2.99	R	0.997	R	
67	22065	2.69	R	0.984	R	
68	22072	2.55	NR	0.726	R	C, NR - R
69	12494	1.50	NR	0.625	R	C, NR - R
70	12514	3.07	R	0.724	R	
71	12493	2.59	R	0.850	R	
72	5833	2.61	R	0.692	R	
73	12520	2.85	R	0.814	R	
74	12528	3.36	R	0.917	R	
75	12524	2.01	NR	0.640	R	C, NR - R
76	22100	4.48	R	1.189	R	
77	22098	2.75	R	0.838	R	
78	22078	2.80	R	1.069	R	

R – risk, NR – no risk, C – change

Appendix 9.21 (continued) A comparison of CV risk estimation using the Roche diagnostic direct LDLC (dLDLC) and the sdLDLC

Sequential number	Lab ID	Roche Diagnostics LDLC (dLDLC)		SdLDLC		Change in risk category/status
		value	risk category	value	risk (R) or no risk (NR)	
79	22106	4.76	R	1.283	R	
80	22105	4.12	R	1.096	R	
81	12561	2.83	R	0.627	R	
82	12552	2.81	R	0.837	R	
83	22142	2.64	R	1.147	R	
84	22140	3.29	R	0.711	R	
85	22143	2.74	R	0.688	R	
86	22144	3.45	R	0.737	R	
87	22231	1.31		1.891		Excluded sample
88	22191	3.32	R	0.694	R	
89	22152	1.26	NR	0.565	R	C, NR - R
90	22155	3.42	R	1.499	R	
91	22157	2.53	NR	0.557	R	C, NR - R
92	22207	5.68	R	1.840	R	
93	22208	3.71	R	1.066	R	
94	22230	1.99	NR	0.535	R	C, NR - R
95	22206	5.08	R	1.234	R	
96	22189	3.10	R	0.731	R	
97	22237	3.08	R	1.199	R	
98	22218	2.37	NR	1.075	R	C, NR - R
99	22188	2.34	NR	0.489	NR	
100	22167	2.78	R	0.570	R	
101	22164	3.27	R	1.022	R	
102	22168	3.30	R	1.526	R	
103	22171	1.95	NR	0.416	NR	
104	22187	1.73	NR	0.265	NR	
105	22186	1.72	NR	0.227	NR	
106	22184	3.56	R	1.041	R	
107	22267	2.90	R	1.084	R	
108	22334	2.16	NR	0.346	NR	
109	22352	2.58	NR	0.705	R	C, NR - R
110	22323	2.60	R	0.706	R	
111	22322	2.53	NR	0.690	R	C, NR - R
112	22351	2.88		2.382		Excluded sample
113	22360	2.53	NR	0.534	R	C, NR - R
114	22335	4.95	R	1.290	R	
115	22307	1.60	NR	0.383	R	C, NR - R
116	22308	2.74	R	1.178	R	
117	22320	2.91	R	0.611	R	

R – risk, NR – no risk, C – change

Appendix 9.21 (continued) A comparison of CV risk estimation using the Roche diagnostic direct LDLC (dLDLC) and the sdLDLC

Sequential number	Lab ID	Roche Diagnostics LDLC (dLDLC)		SdLDLC		Change in risk category/status
		value	risk category	value	risk (R) or no risk (NR)	
118	22318	3.06	R	0.697	R	
119	22330	3.66	R	1.235	R	
120	22327	2.34	NR	0.638	R	C, NR - R
121	22328	3.52	R	1.563	R	
122	12577	3.51	R	1.186	R	
123	12589	3.33	R	0.840	R	

R – risk, NR – no risk, C – change

Appendix 9.22 A comparison of CV risk estimation using the sdLDLC and the non – HDLC

Sequential number	Lab ID	non-HDLC		(sdLDLC)		Change in risk category/status
		value	risk category	value	risk category	
1	36301	4.37	R	0.819	R	
2	10038	6.29	R	1.589	R	
3	5231	4.74	R	1.046	R	
4	10309	5.44	R	1.400	R	
5	960	4.13	R	0.947	R	
6	10186	6.56	R	1.521	R	
7	5191	3.24	NR	0.548	R	C, NR - R
8	10775	4.27	R	0.829	R	
9	10793	4.49	R	1.005	R	
10	10276	5.21	R	1.092	R	
11	10992	3.16	NR	0.588	R	C, NR - R
12	85	4.2	R	0.800	R	
13	10853	4.57	R	0.913	R	
14	5055	1.74	NR	0.260	NR	
15	10908	2.52	NR	0.424	NR	
16	10837	3.77	R	0.980	R	
17	5144	2.77	NR	0.360	NR	
18	10005	3.46	R	0.754	R	
19	10269	5.43	R	1.121	R	
20	5108	5.37	R	1.342	R	
21	5247	2.3	NR	0.414	NR	
22	10418	2.07	NR	0.376	NR	
23	389	2.53	NR	0.504	R	C, NR - R
24	5260	2.5	NR	0.602	R	C, NR - R
25	36300	2.19	NR	0.392	NR	
26	5109	4.15	R	0.683	R	
27	10282	4.19	R	0.896	R	
28	10068	1.44	NR	0.120	NR	
29	36279	4.04	R	0.692	R	
30	10774	4.72	R	0.959	R	
31	35913	3.67	R	0.574	R	
32	10656	1.54	NR	0.197	NR	
33	36169	4.3	R	0.855	R	
34	5161	4.92	R	0.941	R	
35	10295	4.02	R	0.675	R	
36	10792	5.04	R	1.011	R	
37	36135	2.76	NR	0.415	NR	
38	10340	3.02	NR	0.549	R	C, NR - R
39	36061	5.02	R	0.980	R	

R – risk, NR – no risk, C – change

Appendix 9.22 (continued) A comparison of CV risk establishment using the sdLDLC and the non – HDLC

Sequential number	Lab ID	non-HDLC		(sdLDLC)		Change in risk category/status
		value	risk category	value	risk category	
40	1759	2.32	NR	0.420	NR	
41	12023	5.99	R	1.421	R	
42	1988	3.48	R	0.721	R	
43	1798	4.53	R	0.985	R	
44	11587	3.93	R	0.920	R	
45	5491	5.91	R	1.312	R	
46	11826	1.73	NR	0.273	NR	
47	11814	4.17	R	0.968	R	
48	11635	2.38	NR	0.429	NR	
49	11813	2.89	NR	0.573	R	C, NR - R
50	11827	3.12	NR	0.658	R	C, NR - R
51	12414	3.80	R	0.851	R	
52	12423	3.34	NR	0.624	R	C, NR - R
53	12229	4.80	R	1.156	R	
54	22110	2.55	NR	0.438	NR	
55	22099	4.08	R	1.353	R	
56	22091	3.51	R	0.644	R	
57	22088	4.91	R	1.212	R	
58	22087	4.82	R	1.191	R	
59	22077	4.70	R	1.363	R	
60	22034	5.05	R	1.730	R	
61	22063	2.20	NR	0.284	NR	
62	22042	3.28	NR	0.554	R	C, NR - R
63	22041	3.22	NR	0.653	R	C, NR - R
64	22071	7.24	R	1.914	R	
65	22066	3.47	R	0.649	R	
66	22064	3.90	R	0.997	R	
67	22065	3.83	R	0.984	R	
68	22072	3.32	NR	0.726	R	C, NR - R
69	12494	2.47	NR	0.625	R	C, NR - R
70	12514	3.38	R	0.724	R	
71	12493	3.52	R	0.850	R	
72	5833	3.13	NR	0.692	R	C, NR - R
73	12520	3.23	NR	0.814	R	C, NR - R
74	12528	4.11	R	0.917	R	
75	12524	2.48	NR	0.640	R	C, NR - R
76	22100	5.08	R	1.189	R	
77	22098	3.32	NR	0.838	R	C, NR - R
78	22078	3.76	R	1.069	R	

R – risk, NR – no risk, C – change

Appendix 9.22 (continued) A comparison of CV risk establishment using the sdLDLC and the non – HDLC

Sequential number	Lab ID	non-HDLC		(sdLDLC)		Change in risk category/status
		value	risk category	value	risk category	
79	22106	4.94	R	1.283	R	
80	22105	4.84	R	1.096	R	
81	12561	3.65	R	0.627	R	
82	12552	4.18	R	0.837	R	
83	22142	4.39	R	1.147	R	
84	22140	4.62	R	0.711	R	
85	22143	3.96	R	0.688	R	
86	22144	4.65	R	0.737	R	
87	22231	4.18		1.891		Excluded sample
88	22191	4.23	R	0.694	R	
89	22152	2.25	NR	0.565	R	C, NR - R
90	22155	5.33	R	1.499	R	
91	22157	3.38	R	0.557	R	
92	22207	8.15	R	1.840	R	
93	22208	5.11	R	1.066	R	
94	22230	2.79	NR	0.535	R	C, NR - R
95	22206	6.60	R	1.234	R	
96	22189	4.14	R	0.731	R	
97	22237	5.32	R	1.199	R	
98	22218	3.78	R	1.075	R	
99	22188	3.06	NR	0.489	NR	
100	22167	3.60	R	0.570	R	
101	22164	4.71	R	1.022	R	
102	22168	5.54	R	1.526	R	
103	22171	2.80	NR	0.416	NR	
104	22187	2.37	NR	0.265	NR	
105	22186	2.26	NR	0.227	NR	
106	22184	5.20	R	1.041	R	
107	22267	4.28	R	1.084	R	
108	22334	2.69	NR	0.346	NR	
109	22352	3.74	R	0.705	R	
110	22323	3.64	R	0.706	R	
111	22322	3.75	R	0.690	R	
112	22351	5.18		2.382		Excluded sample
113	22360	3.19	NR	0.534	R	C, NR - R
114	22335	6.40	R	1.290	R	
115	22307	2.48	NR	0.383	NR	
116	22308	4.32	R	1.178	R	
117	22320	3.79	R	0.611	R	

R – risk, NR – no risk, C – change

Appendix 9.22 (continued) A comparison of CV risk establishment using the sdLDLC and the non – HDLC

Sequential number	Lab ID	non-HDLC		(sdLDLC)		Change in risk category/status
		value	risk category	value	risk category	
118	22318	3.87	R	0.697	R	
119	22330	5.15	R	1.235	R	
120	22327	3.52	R	0.638	R	
121	22328	5.08	R	1.563	R	
122	12577	5.25	R	1.186	R	
123	12589	3.62	R	0.840	R	

R – risk, NR – no risk, C – change

Appendix 9.23 cLDLC and dLDLC levels paired t test

T-Test calculator

Result

You entered the following data:

Group 1																	Group 2
3.99	5.08	4	3.79	3.41	5.94	2.84	4.07	3.88	4.58	2.79	3.6	4.69	3.65	3.17	3.23	5.65	2.5
3.79	3.99	1.51	2.22	2.6	2.68	2.66	5.02	4.31	1.88	1.75	3.44	3.55	1.58	2.13	2.4	2.43	2.3
2.21	1.78	1.91	3.79	3.24	1.16	3.5	4.19	3.41	1.26	3.73	2.29	1.82	1.97	3.23	2.73	1.05	2.9
4.42	3.52	4.51	2.48	2.49	4.4	1.94	5.28	3.05	4.05	3.11	3.88	2.92	4.04	2.23	2.21	3.8	1.9
5.46	1.34	3.62	2.09	2.59	2.69	3.51	2.14	4.27	2.19	2.7	5.21	1.33	3.59	2.11	2.62	2.68	3.6
3.22	4.21	4.18	3.38	3.14	1.94	2.8	2.54	5.73	3.09	3.02	3.03	4.18	4.2	3.39	3.37	1.75	2.4
2.81	2.68	1.54	3.03	2.69	2.61	2.66	3.51	1.82	4.55	2.64	2.69	2.55	1.5	3.07	2.59	2.61	2.8
2.7	4.47	4.28	3.24	3.36	3	4.16	3.35	4.24	3.89	1.31	2.8	4.76	4.12	2.83	2.81	2.64	3.2
3.71	2.95	6.78	4.3	2.2	6.04	3.64	3.92	2.46	2.68	3.25	3.42	2.53	5.68	3.71	1.99	5.08	3.1
3.79	3.77	2.35	2.09	2.05	4.32	3.15	2.48	3.06	2.99	3.06	3.27	3.3	1.95	1.73	1.72	3.56	2.9
2.83	5.73	1.94	2.96	3.42	3.45	4.07	2.84	3.48	4.13	3.21	2.53	4.95	1.6	2.74	2.91	3.06	3.6

The means of Group 1 and Group 2 are significantly different at p < 0.01.

Summary	Group 1		Group 2
Mean	3,2868		3,0158
Variance	1.1605		0.9416
Stand. Dev.	1.0773		0.9704
n	121		121
t		**10,8436**	
degrees of freedom		**120**	
critical value		**2.617**	

Two sample t-test | One sample t-test

Two sample t-test calculator

Enter Data for Group 1

Enter Data for Group 2

Use data grit to input values

1, Group description:

Groups Have Equal Variance (default)

2, Number of tails:

Two Tailed Test (default)

Appendix 9.24 cLDLC and non-HDLCA levels paired t test

T-Test calculator

Result

You entered the following data:

Group 1																Group 2
3.99	5.08	4	3.79	3.41	5.94	2.84	4.07	3.88	4.58	2.79	3.59	5.51	4.01	4.66	3.35	5.78
3.79	3.99	1.51	2.22	2.6	2.68	2.66	5.02	4.31	1.88	1.75	3.42	3.79	0.96	1.74	2.99	1.99
2.21	1.78	1.91	3.79	3.24	1.16	3.5	4.19	3.41	1.26	3.73	1.75	1.72	1.41	3.37	3.41	0.66
4.42	3.52	4.51	2.48	2.49	4.4	1.94	5.28	3.05	4.05	3.11	4.14	3.24	4.26	1.98	2.24	4.24
5.46	1.34	3.62	2.09	2.59	2.69	3.51	2.14	4.27	2.19	2.7	5.13	0.95	3.39	1.6	2.11	2.34
3.22	4.21	4.18	3.38	3.14	1.94	2.8	2.54	5.73	3.09	3.02	2.73	4.13	4.04	3.92	4.27	1.42
2.81	2.68	1.54	3.03	2.69	2.61	2.66	3.51	1.82	4.55	2.64	3.05	2.54	1.69	2.6	2.74	2.35
2.7	4.47	4.28	3.24	3.36	3	4.16	3.35	4.24	3.89	1.31	2.98	4.16	4.06	2.87	3.4	3.61
3.71	2.95	6.78	4.3	2.2	6.04	3.64	3.92	2.46	2.68	3.25	4.55	2.6	7.37	4.33	2.01	5.82
3.79	3.77	2.35	2.09	2.05	4.32	3.15	2.48	3.06	2.99	3.06	3.93	4.76	2.02	1.59	1.48	4.42
2.83	5.73	1.94	2.96	3.42	3.45	4.07	2.84	3.48	4.13	3.21	2.41	5.62	1.7	3.54	3.01	3.09

The means of Group 1 and Group 2 are significantly different at p < 0.01.

Summary	Group 1		Group 2
Mean	3.2868		3.1685
Variance	1.1605		1.4968
Stand. Dev.	1.0773		1.2234
n	121		121
t		**3.3984**	
degrees of freedom		**120**	
critical value		**2.617**	

Two sample t-test | One sample t-test

Two sample t-test calculator

Enter Data for Group 1

Enter Data for Group 2

Use data grit to input values

1. Group description:

- Groups Have Equal Variance (default)
- Groups Have Unequal Variance (Welch t-test)

2. Number of tails:

- Two Tailed Test (default)
- One Tailed Test

3. Significance Level:

4. Choose a test

Appendix 9.25 dLDLC and non-HDLCA levels paired t test

T-Test calculator

Result

You entered the following data:

Group 1											Group 2					
3.6	4.69	3.65	3.17	3.23	5.65	2.51	3.9	3.69	4.12	2.63	3.59	5.51	4.01	4.66	3.35	5.78
3.44	3.55	1.58	2.13	2.4	2.43	2.39	4.65	4.03	1.82	1.82	3.42	3.79	0.96	1.74	2.99	1.99
2.29	1.82	1.97	3.23	2.73	1.05	2.9	3.8	2.97	1.27	3.33	1.75	1.72	1.41	3.37	3.41	0.66
3.88	2.92	4.04	2.23	2.21	3.8	1.91	5.05	2.96	3.88	2.92	4.14	3.24	4.26	1.98	2.24	4.24
5.21	1.33	3.59	2.11	2.62	2.68	3.63	1.34	4.3	2.07	3.04	5.13	0.95	3.39	1.6	2.11	2.34
3.03	4.18	4.2	3.39	3.37	1.75	2.4	2.27	5.29	2.87	2.99	2.73	4.13	4.04	3.92	4.27	1.42
2.69	2.55	1.5	3.07	2.59	2.61	2.85	3.36	2.01	4.48	2.75	3.05	2.54	1.69	2.6	2.74	2.35
2.8	4.76	4.12	2.83	2.81	2.64	3.29	2.74	3.45	3.32	1.26	2.98	4.16	4.06	2.87	3.4	3.61
3.42	2.53	5.68	3.71	1.99	5.08	3.1	3.08	2.37	2.34	2.78	4.55	2.6	7.37	4.33	2.01	5.82
3.27	3.3	1.95	1.73	1.72	3.56	2.9	2.16	2.58	2.6	2.53	3.93	4.76	2.02	1.59	1.48	4.42
2.53	4.95	1.6	2.74	2.91	3.06	3.66	2.34	3.52	3.51	3.33	2.41	5.62	1.7	3.54	3.01	3.09

The means of Group 1 and Group 2 are significantly different at p < 0,01.

Summary	Group 1		Group 2
Mean	3,0158		3,1685
Variance	0,9416		1,4968
Stand. Dev.	0,9704		1,2234
n	121		121
t		**-3,3987**	
degrees of freedom		**120**	
critical value		**2,617**	

Two sample t-test | One sample t-test

Two sample t-test calculator

Enter Data for Group 1

Enter Data for Group 2

Use data grit to input values

1. Group description:

- Groups Have Equal Variance (default)
- Groups Have Unequal Variance (Welch t-test)

2. Number of tails:

- Two Tailed Test (default)
- One Tailed Test

3. Significance Level:

4. Choose a test

Appendix 9.26 HDLC/cLDLC and HDLC/dLDLC values paired t test

Result

You entered the following data:

Group 1												Group 2									
0.3	0.3	0.2	0.1	0.3	0.2	0.5	0.5	0.4	0.2	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.6	0.6	0.4	0.3	0.4
0.2	0.3	1	0.5	0.4	0.6	0.3	0.2	0.2	0.7	0.6	0.3	0.3	0.9	0.6	0.4	0.7	0.4	0.3	0.2	0.8	0.6
0.9	0.5	1.1	0.5	0.5	1.1	0.4	0.3	0.4	1.2	0.4	0.8	0.5	1	0.5	0.6	1.2	0.5	0.4	0.5	1.2	0.4
0.2	0.4	0.3	0.5	0.4	0.3	0.7	0.3	0.6	0.3	0.3	0.2	0.4	0.3	0.6	0.5	0.3	0.8	0.4	0.6	0.4	0.3
0.3	1	0.3	1	0.6	0.5	0.4	0.2	0.4	0.5	0.3	0.3	1	0.3	1	0.6	0.5	0.4	0.3	0.3	0.6	0.3
0.4	0.3	0.3	0.3	0.3	0.5	0.3	0.3	0.2	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.6	0.4	0.3	0.2	0.3	0.3
0.4	0.4	0.7	0.4	0.7	0.7	0.5	0.4	1	0.2	0.8	0.4	0.4	0.7	0.4	0.7	0.7	0.5	0.4	0.9	0.2	0.7
0.4	0.5	0.2	0.3	0.4	0.4	0.4	0.3	0.3	0.4	0.9	0.3	0.4	0.2	0.4	0.5	0.4	0.6	0.4	0.4	0.4	0.9
0.2	1.1	0.1	0.3	0.7	0.3	0.4	0.2	0.6	0.5	0.5	0.2	1.2	0.1	0.4	0.8	0.3	0.5	0.3	0.6	0.6	0.6
0.4	0.2	0.3	0.4	0.5	0.2	0.3	0.6	0.3	0.4	0.3	0.4	0.2	0.4	0.5	0.6	0.3	0.3	0.7	0.3	0.5	0.3
0.5	0.1	0.4	0.2	0.5	0.4	0.3	0.3	0.3	0.3	0.4	0.5	0.2	0.5	0.2	0.6	0.5	0.3	0.3	0.3	0.3	0.4

The means of Group 1 and Group 2 are significantly different at p < 0.01.

Summary			
	Group 1		**Group 2**
Mean	0.4306		0.4645
Variance	0.0526		0.0506
Stand. Dev.	0.2293		0.2249
n	121		121
t		**-6.0804**	
degrees of freedom		**120**	
critical value		**2.617**	

Explanation

Step 1: Find t value and **degrees of freedom**

To find t value and degrees of freedom we will use following formulas:

$$t = \frac{\overline{X_D}}{\frac{S_D}{\sqrt{n}}}$$

$$d.o.f = n - 1$$

$\overline{X_D}$ = Mean of differences between pairs
S_{X_D} = Standard deviation of differences between pairs
$d.o.f$ = degrees of freedom
n = Total number of values in first(second) dataset

In this example we have:

$$\overline{X_D} \approx -0.0339$$

$$S_{X_D} = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(X_{Di} - \overline{X_D})^2} \approx 0.0613$$

After substituting these values into the formula for t we have:

$$t = \frac{\overline{X_D}}{\frac{S_D}{\sqrt{n}}} = \frac{-0.0339}{\frac{0.0613}{\sqrt{121}}} \approx -6.0804$$

The degrees of freedom is:

$$d.o.f = n - 1 = 120$$

Step 2: Determine critical value for t with degrees of freedom = 120 and $\alpha = 0.01$.

In this example the critical value is 2.617 (see the table below).